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Spatial and competition effects in tree breeding

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Abstract Book

INRA SCIENCE & IMPACT université de BORDEAUX cirad IUFRO

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Welcome to the IUFRO 'Genomics and Forest Genetics' Conference

For the first time this conference brought together 4 working groups of IUFRO sub-division 2.4 'Genetics' in a single event.

2.04.01 - Population, ecological and conservation genetics
2.04.02 - Breedingtheory and progenytesting
2.04.06 - Molecularbiology of foresttrees
2.04.10 - Genomics

A general unifying theme of theseworking groups is the application of molecular technologies to study important commercial or evolutionary traits of forest trees. The traits of study can range from expression of a single gene in a particular tissue to allele frequencies among hundreds of genes in populations that span a continent. Information from the smallest scale of investigation can often be used in investigations at the largest scales. With this in mind the Genetics Research Group provides an organizational structure where ideas and information are openly exchanged among seemingly disparate fields of research.

The objective of the conference is to present and discuss new scientific findings in the area of population, quantitative and evolutionary genetics and how they can be applied in genetic resource conservation and breeding**.** It is organized by INRA, Bordeaux University, CIRAD and IUFRO and has over 250 attendees from 35 countries.

The conference will take place in Arcachon (France), at the gate of the Atlantic Ocean just 1 hour from Bordeaux. The scientific programme is extremely busy with 73 talks and 140 posters, but we hope you can enjoy the city and the surrounding.

Thank you for attending the conference and we hope you have a great time with us.

Christophe Plomion
Jean-Marc Gion
Francis Martin
Antoine Kremer

The conference is dedicated to the Memory of two distinguished forest tree geneticists: **Jean-François Lacaze**(INRA, France) and **Thomas Ledig** (UC Davis, United States).

Presentation Abstracts

OPENING KEYNOTE LECTURE: **Outi Savolainen**

Genomic consequences of phenotypic selection (in *Pinus sylvestris* and *Arabidopsis lyrata*)
Outi Savolainen, Jaakko Tyrmi, Tiina Mattila, Sonja Kujala, Katri Kärkkäinen
Plant population genetics, University of Oulu, Finland

Conifers are known to experience strong selection in different life stages. Extensive provenance trials and other experiments have demonstrated adaptation to local climatic conditions, as seen in patterns of phenotypic variation correlated with climatic conditions. On the other hand, after selfing, there is high mortality at especially early life stages due to the high numbers of lethal equivalents. Current theories make predictions on the expected patterns of variation at loci related to local adaptation. Further, the effects of selection due deleterious recessives on genomic patterns of variation have also been predicted. Initial genetic data from exome capture in Scots pine and whole genome sequences in the outcrossing perennial Arabidopsis lyrata can be contrasted against some of these predictions.

SESSION 1

KEY NOTE: Nicolas Bierne

Crossing the species barrier: is local interspecies introgression adaptive?
Nicolas Bierne
CNRS, France

Although introgression -the flow of genes between partially isolated genetic backgrounds- is under study for decades, the qualitative and quantitative importance of introgression in evolution is still discussed. The debate has recently been revivified with genomics data that revealed the ubiquity of introgression in many systems including our own species, and many trees. Many open questions persist about introgression: How long species barriers remain permeable? Why are some regions of the genome less resistant to introgression than others? Is adaptive introgression really so widespread? Could it simply be a spectacular manifestation of the general process of adaptation which nonetheless mainly proceeds by intra-specific evolution? Could the pattern of local introgression be attributed to other phenomena than trans-specific positive selection? I will try to offer some elements of answer to these questions in the light of theoretical arguments from the Fisher's geometric model and of experimental works in my study systems, marine invertebrates, which are not trees but share many similarities with trees.

Genomic architecture of adaptation and species boundaries in a Eurasian Populus species complex
Camille Christe1, Kai N. Stölting1, Margot Paris1, Luisa Bresadola1, Christelle Fraisse23, Dorothea Lindtke14, Celine Caseys1, Daniel Wegmann1, Nicolas Bierne23, Alex Buerkle4, **Christian Lexer**15*
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Rapid recent progress in ecological & evolutionary genomics is imparting fresh perspectives to the study of adaptation and speciation, i.e. the origin and maintenance of biological diversity. Evolutionary genomic studies of the genus Populus (poplars, aspens, cottonwoods) have already contributed greatly to our current understanding of these topics, facilitated by the early availability of a functionally annotated chromosome-level genome assembly. We will highlight recent progress of our ongoing

research on the genomic architecture of adaptation and species boundaries along the divergence continuum in Eurasian Populus spp. We will explore the determinants of divergence and cohesion along this continuum, ranging from differentiated populations and phylogeographic lineages to highly divergent species with incomplete reproductive barriers (e.g. Genetics 186:699, 2010; Heredity 111:474, 2013; Molecular Ecology, 22:842, 2013; Ecology Letters 16:1515, 2013; Molecular Ecology 23: 4316, 2014; New Phytologist 207: 723-734, 2015; PLoS ONE 2015, 10: e0128200). By coupling genomics with experiments, we are beginning to understand how the genomic landscape of divergence is transformed during the process of speciation, which isolating mechanisms are involved in making this possible, and how genetic interactions between previously diverged species affect the functionally important genetic variation (e.g. for chemical defence traits) present in these ecologically important foundation species. To illustrate our points, we will present results from recent whole genome sequencing (WGS) and reduced complexity library sequencing (RAD and GBS) studies of hybrid zones between the two wide-spread taxa Populus alba (White poplar) and P. tremula (European aspen). In these highly divergent species, model-free and model-based approaches reveal the genomic footprint of a complex joint demographic history with recurrent gene flow episodes. Nevertheless, species integrity is maintained by strong postzygotic selection, consistent with the 'genomic coadaptation' model of barriers in secondary contact (Molecular Ecology 2016, doi: 10.1111/mec.13587). Based on recent results on hybrid survivorship and genomic patterns of adaptive and deleterious coding mutations, we will discuss the potential role of heterosis in facilitating both, F1 hybrid persistence and the episodic breakdown of barriers between two species that apparently diverged for millions of years.

Identification of recent secondary contacts between four morphologically distinct oak species

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*Presenting author

An important issue in evolutionary biology is to understand how historical events have shaped the diversity currently observed within species. Migration between gene pools is a major force homogenizing genomes at neutral loci, but it can also reveals loci acting as species barriers by decreasing the viability or fertility of produced hybrids.Here, we quantify past and current events of gene flow between four morphologically divergent oak species (Quercus petraea, Q. robur, Q. pyrenaica, Q. pubescens) by using two independent inference methods: diffusion approximation to the joint frequency spectrum and Approximate Bayesian Computation. For each pairs of species, alternative scenarios of speciation allowing gene flow at different timescales were evaluated. Based on 3,524 SNPs detected in gene sequences spanning the genome, we found that current introgression between oak species is the result of an unambiguous secondary contact following a long period of isolation, probably as the result of the last postglacial recolonization.Based on the inferred secondary contact with genomic heterogeneity in gene flow, and the different ecological requirements between these white oak species (e.g. soil pH or moisture), we subsequently performed a whole-genome scan for divergence (along the 12 pseudo-chromosomes of the reference oak genome sequence) to identify the reproductive barriers maintaining species integrity in the face of interspecific gene flow. Allele frequencies were estimated based on deep pool sequencing for each of the four species (up to 450x genome coverage per species). We will report evidence for narrow regions of high divergence and candidate speciation genes for both intrinsic and ecological barriers including genes involved in flowering or drought tolerance.

Genome-wide local ancestry analysis reveals cassette-like adaptive introgression from Populus balsamifera (balsam poplar) into P. trichocarpa (black cottonwood)

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Natural hybrid zones in forest trees provide systems to study the transfer of adaptive genetic variation by introgression. We used population-wide whole genome resequencing data from the North American sister species Populus trichocarpa and P. balsamifera to investigate the role of hybridization and introgression in local adaptation in these closely related yet ecologically divergent species. Landscape genomic studies in P. trichocarpa indicated genomic footprints of admixture with P. balsamifera, with admixed individuals mostly restricted to drainages at the edge of the P. trichocarpa distribution near contact with the range of P. balsamifera (Geraldes et al., 2014), consistent with a “porous” or “semipermeable” species boundary (Harrison and Larson, 2014). Using Fst outlier (Geraldes et al., 2014) and GWAS (McKown et al., 2014) approaches, with extensive phenotypic data, we identified P. trichocarpa candidate genes for local adaptation. To identify introgressed regions at a fine genomic scale, we employed local ancestry analysis using whole genome resequencing data from pure species and admixed individuals. In admixed P. trichocarpa individuals, ancestry analysis revealed 25 genomic regions of P. balsamifera ancestry on 12 chromosomes. In contrast, admixed P. balsamifera individuals contained 10 distinct genomic regions of P. trichocarpa ancestry on 6 chromosomes. These data are consistent with a “semipermeable” species boundary (Harrison and Larson, 2014), allowing differential introgression of certain alleles across the boundary, some of which could be the result of selective processes. To test the hypothesis of cassette-like adaptive introgression, we focused on a 580-kb telomeric region in chromosome 15 of P. balsamifera ancestry present in admixed P. trichocarpa individuals from the northern and north-central extremes of its range, which contains several candidate genes for local adaptation (e.g. PRR5, COMT1, TTG1; Geraldes et al., 2014; McKown et al., 2014). In contrast, a paralogous block of genes in chromosome 12 showed no signs of introgression or signatures of selection. Genomic analyses revealed signals of selection in certain genes in the chromosome 15 region, and functional analyses based on gene expression variation and correlations with adaptive phenotypes suggest distinct functions of the introgressed alleles (Suarez-Gonzalez et al., 2016). We hypothesize that the introgressed region in chromosome 15 is an example of differential, adaptive introgression that has introduced modular, cassette-like variation into P. trichocarpa individuals, adapting them to transitional environments at the extremes of this species’ range. The linked adaptive mutations are in genes

Landscape genomics approach to study historic response of mountain hemlock to Pleistocene glaciation at its range limit in Alaska, USA

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Quaternary glaciations were very important processes structuring present day northern vegetation. In many cases, advancing glaciers forced species to retreat into southern portions of their range, where climates were more suitable, or reduced distribution into microrefugia, where climate remained within the species tolerance. As glaciers receded, species slowly recolonized their previous northern distributions. However, because of the potential for alternative recolonization pathways, discerning the history of species distribution and migration is often difficult. Even when species are distributed in geographically isolated patches, suggesting pre-glaciation refugia, we cannot discount the ability of rare long distance dispersal (LDD) to establish them. We used molecular ecological and landscape

genomics approaches to address two research questions: 1) Are isolated stands of mountain hemlock found in the Kenai lowlands glacial relicts or are they the product of LDD following glacial retreat? 2) What is the degree to which mountain hemlock patches across the Kenai Peninsula have been historically connected by gene-flow? To address these questions we used SNP markers discovered in double digest Restriction Associated DNA sequencing (ddRADseq) of multiple trees collected from eight mountain hemlock sample sites on the Kenai Peninsula, Alaska USA. We found significant differences in genomic diversity (P = 0.003) and genetic structure (P = 0.03) between isolated stands of mountain hemlock and those found across the rest of the peninsula. A graph approach based on electrical circuit analysis identified high landscape connectivity and conductance across the peninsula. Genetic variation (22 %) was primarily explained by landscape resistance (r = 0.469, P = 0.103) and not geographic distance (r = 0.293, P = 0.153). These findings suggest that mountain hemlock colonized the peninsula via LDD and repeated founding events accompanied by high levels of gene-flow.

KEY NOTE: Alex Buerkle

Inconsistent reproductive isolation

Alex Buerkle
University of Wyoming, United States

Speciation has commonly been studied as if variable genetics, phenotypes and environments of species were unlikely to affect reproductive isolation between species. Perhaps unsurprisingly, for taxa that are incompletely isolated, this simplification is probably commonly inappropriate and has the potential to mislead us about the nature of barriers to reproduction between species. I will discuss several examples of diversity of reproductive isolation in plants and animals, and the implications these have for thinking about species and boundaries between them.

Living-at-the-Edge: Speciation and Adaptation in Marginal Populations of two Mediterranean White Oaks

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Marginal populations have received much attention during the last years, especially since their contribution to species genetic diversity has been properly recognized. Marginal populations usually carry adaptations to harsh environments where most populations from such species could not survive, thus becoming the focus of conservation efforts on the one hand, and evolutionary analyses on the other. In this study, we analyzed the genome-wide structure (110 EST-SSRs) of the genetic diversity and differentiation in 4 inter-specific pairs of marginal populations from two Mediterranean white oaks, Quercus faginea and Q. pyrenaica. Population pairs were selected along 6 latitudinal degrees (37-42°), from the edges of the distribution areas or from places under extreme abiotic (e.g., summer temperature) or biotic (e.g., long-term isolation) conditions. Most sampled individuals (> 98%) presented clear leave-morphology differences that allowed an easy species discrimination. Bayesian clustering indicated that 3 inter-specific population pairs formed one distinct genetic group each. Thus, it was not surprising that overall genetic differentiation among-populations within-species was much higher than overall between-species differentiation. Inter-specific genome scans suggested GOT021 (a member of the HK gene-family that is involved in cytokinin reception, HK4-like) could be responsible for geographic-independent speciation. Other regions previously identified by their high inter-specific differentiation (LGs#2 and 12) probably take part in the geographic mosaic of speciation. Intra-specific genome scans identified mostly the same outliers among populations from the two species. Evidence of their involvement in local adaptation was further obtained from the functional annotation of ESTs with significant genomic-environmental associations. Our results could be explained by either selection of ancestral polymorphisms or by a history of gene flow and admixture between incipient species that allows the interchange of selected loci. Coalescent simulations using SSRs were able to differentiate between several admixture scenarios (Sousa et al., 2012), thus suggesting they could be helpful to discriminate between our two hypotheses. If the latest were the case, the Mediterranean white oaks would fit into the “reciprocal evolutionary change” strict coevolution definition, thus being eligible for a

rarely discussed case of mutualism that is characterized by convergent coevolution.

Comparative population genomics of local adaptation

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Environmental conditions can vary abruptly within a landscape. When continuous tree stands occur across environmental gradients or contrasts, populations can undergo microgeographic1 adaptation (i.e. adaptive divergence within individual dispersal range), arising from the interplay of dispersal, gene flow, and selection. If environmental variation affects intra-populational genetic diversity, this may partly explain the maintenance of adaptive, and associated neutral, variability and adaptive potential. In spite of growing evidence in favour of microgeographic adaptation in trees2, and of an increasingly coherent theoretical and modelling framework describing the conditions of existence of such a process3, information on its genome-wide effects is scant.We have undertaken the study of the extent of sub-population divergence along local environmental gradients through sequence capture in eight tree species (Abies alba, Cedrus atlantica, Eperua falcata, Fagus sylvatica, Larix decidua, Pinus halepensis, Pinus pinaster, and Symphonia globulifera), chosen to cover a wide variety of ecosystems, from alpine to mediterranean to tropical. For each species, one to three pairs of sub-populations, representative of similar ecological gradients but distant enough from each other to be considered as independent, were studied. Because the subpopulations belonged to continuous stands and had minimal (sometimes zero) genome-wide neutral divergence, our experimental design was ideally suited to detect weak signals of ecological-gradient related divergence at individual loci.We obtained between six thousand and twelwe thousand SNPs from approximately twelwe thousand independent sequences for each species. Within each species and each pair of subpopulations, between 0.5% and 5% (depending on detection method and stringency) of the polymorphic loci were divergence outliers (and therefore candidates for directional or disruptive selection). This result suggests that intra-populational, microgeographic adaptive processes can be strong4,5,6, even though gene flow should tend to wipe out patterns induced by selection at such short geographical distances. Overlap of lists of divergent loci across same-species subpopulation pairs was limited. This may be a consequence of limited statistical power, but may also suggest that different genotypic combinations are selected in different populations, or that slight differences in local environmental conditions along similar gradients lead to different patterns of population divergence.The results will be discussed in the light of current knowledge about mechanisms maintaining intraspecific and intra-populational genetic diversity, and in the context of the assessment of natural functional diversity and adaptive potential to global change.

Gene-specific Introgression drives local climatic adaptation in a North American spruce hybrid zone (Picea engelmannii x P. glauca)

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*Presenting author

Engelmann spruce (Picea engelmannii) and white spruce (Picea glauca) are closely-related North American conifers that occupy distinct ecological niches in temperate and boreal regions, respectively. These species hybridize extensively throughout western Canada in environments intermediate to those of the parent species'. There is some evidence that hybrids have superior fitness in these environments. Climatic modeling suggests that this hybrid zone is ancient, and its current climatic and spatial extents are among the broadest described, spanning at least 285,000 km2. This affords a unique opportunity to study the long-term genomic effects of hybridization on a massive environmental scale. Additionally, 32% of the multivariate climatic niche envelope of hybrids does not overlap with the niche envelopes of either parent species, suggesting that hybridization has allowed for colonization of novel habitats. As part of the AdapTree Large-Scale Applied Genomics Project, sequence capture data was generated for ~23,000 genes from 572 individuals representing 254 natural populations of both parental species and their hybrids across western Canada. A panel of 14,009 SNPs exhibiting allele frequency differences greater than 0.6 between species was selected for genomic cline analysis using bgc, identifying 4,944 SNPs across 1,305 genic regions where parental alleles are

disproportionately introgressed (DI) into hybrids. These DI alleles are equally distributed between parent species, although P. engelmannii DI alleles tend to be more strongly introgressed. In genotype-environment associations performed using Bayenv2 across 23 important geoclimatic variables, DI SNPs were more likely to be associated with climatic variables than SNPs exhibiting neutral introgression, suggesting an adaptive signature for DI alleles. A climatic comparison between parental species' habitats and the hybrid zone revealed that hybrid habitats tend to be more similar to one parental species or the other rather than intermediate for many climatic variables. The strength of association between DI alleles and environmental variables is strongly correlated with hybrid-parent environmental similarity for those variables ($R2 = 0.77$, $p < 0.0001$), suggesting that bi-directional adaptive introgression is allowing for fine-scale local adaptation to climate within the hybrid zone. This may help explain the observed hybrid superiority within the hybrid zone, and the apparent ability of hybrids to colonize novel habitats using unique combinations of parent species' climatic adaptations.

Widespread selection and adaptive introgression of parental alleles in the genome of the hybrid pine Pinus densata

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Homoploid hybrid speciation (HHS) is increasingly recognized as an important form of rapid organismal evolution in both plants and animals. Unlike hybrid speciation accompanied by ploidy changes, which can cause near instantaneous reproductive isolation of the incipient hybrids from their progenitors, reproductive isolation in HHS is often facilitated by ecological selection and/or geographical isolation, which promotes the adaptation of the hybrids to a novel habitat. Diverse geographical modes and mechanisms are often involved at different stages of a hybrid speciation history, but genome-wide outcomes of the different evolutionary processes during HHS are less well understood. An important step towards understanding the genome dynamics of HHS is identifying key speciation events and environments and performing comparative genomic analyses in populations of the hybrid relative to the parent populations.Pinus densata forms extensive forest on the Tibetan Plateau and represents one of the most ecologically successful cases of homoploid hybrid speciation in plants. To understand the genetic mechanisms of this speciation, we conducted comparative genomic analysis in populations of P. densata and its two parent species P. tabuliformis and P. yunnanensis by exome capture sequencing and genotyping-by-sequencing. We found accelerated and widespread coding sequence divergence between P. densata and the parent species. Intra- and interspecific levels of sequence diversity and divergence analyses suggest widespread divergent selection in exome sequences, and this pattern is remarkable in the early stages of species diversification. Unlike the distinct mosaic genome structure discovered in other recent hybrid species, the genome of the advanced generation of the P. densata population is more homogenized by introgression from P. yunnanensis, and only 2% of the exome loci are dominated by P. tabuliformis alleles. These alleles in P. densata seem to be maintained by divergent or balancing selection. Our study reveals that introgression, selection and demographic events all had an impact on the genome architecture and diversity of the P. densata population, and highlights the important role of multiple modes of selection on parental genetic variation, mediated by hybridization/introgression, to facilitate the rapid environmental adaptation of hybrid populations. Our findings also shed lights on the genomics of conifer evolution defined by a weak genetic barrier but strong adaptation to local environments.

Evolutionary dynamics of the leaf phenological cycle in an oak metapopulation along an elevation gradient

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*Presenting author

Predictions suggest that climate change will radically alter the selection of phenological traits. Long-lived species, such as trees, will be particularly affected, as they will have only one or a few

generations to track a moving fitness optimum. The traits describing the annual life cycle of trees generally have a high evolutionary potential, but nothing is known about the strength of their genetic correlation. Strong correlations can impose evolutionary constraints, potentially hampering the adaptation of multivariate phenological phenotypes. We investigated the influence of the G-matrix linking leaf unfolding and leaf senescence timing in an oak metapopulation along an elevation gradient (see ref [1]). The study of local and future adaptation to climate has mostly been focused on spring phenology, but recent studies have pointed that fall phenology should not be neglected (e.g. [2, 3]). From a coupled in situ and common garden design, we found that the genetic correlation between these traits was very weak, indicating that adaptation to extreme climatic conditions at high elevations results from correlated selection on both the spring and the fall phenological traits, compensating for the shortening of canopy duration due to delayed bud flushing to cope with colder spring temperatures. A parallel GWAS study of phenological traits found results congruent with the the one obtained from the pedigree information. Comparing the G-matrix with divergence patterns along the gradient showed selection for spring phenology to be twice as strong as for fall phenology. These results highlight the benefits of integrative quantitative genetic investigations of phenological cycles for predicting the capacity of populations to cope with climate change.

SESSION 2

KEY NOTE: Angela Hancock

Understanding local adaptation using Arabidopsis thaliana as a model

Angela Hancock
Molecular basis of adaptative evolution, Max F. Perutz Laboratories

Arabidopsis thaliana is a superb model for studying local adaptation because it is geographically widespread and its range encompasses extensive variation in climate and other environmental factors. I will discuss work we are doing to identify loci and pathways involved in local adaptation across the A. thaliana range as well as efforts to comprehensively characterize population history and adaptation in specific cases. Depending on the details of population history, different models of phenotypic variation and adaptation may be most relevant. Moreover, optimal study design for mapping traits and elucidating adaptive responses depends on population history due to its effects on overall levels of genetic and phenotypic variation, genetic and allelic heterogeneity and the extent of linkage disequilibrium. I will discuss the relevance of these factors to different A. thaliana cases and the more general implications.

Making the most of a high CO2 world: genomic factors associated with eucalypt performance under future CO2 conditions

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The unabated rise in atmospheric CO2 presents a global challenge, with potential to enhance forest productivity and carbon mitigation via CO2 fertilisation effects. Eucalypts are one of the world's most dominant hardwood species, widely planted, and an important foundation species in Australasian forest ecosystems. Current knowledge of eucalypt responses to high CO2 is insufficient to identify which species, and genotypes within species, will be most productive in a high CO2 world. As part of a large multidisciplinary project we are characterising genomic factors underpinning photosynthetic regulation and growth in high CO2 conditions by linking quantitative genomics, leaf-based phenomics and tree physiology. The goal is to deliver diagnostic markers and decision tools to aid selection for improved phenotypic performance across multiple eucalypt species. This work has focused on four key eucalypt species; E. camaldulensis, E. grandis, E. globulus and E. nitens. In each case, phenotypic assessment is being performed on young plants under "high" (640ppm) and "normal"

(400ppm) CO2 in glass house conditions, followed by whole genome association analyses, to identify genetic factors underpinning performance.Here we present findings from the first species, E. c. camaldulensis. An association population consisting of 420 diverse genotypes spanning the natural range of the subspecies was established and maintained as clonal hedges. Detailed phenotyping on a subset of genotypes identified CO2 responsive traits, including height, diameter, stem volume, biomass, leaf area and d13C, which were screened across the population. Variance in these traits was shown to reflect heritable genetic factors (h2: 0.21 to 0.43). A lack of a significant G x CO2 effect suggests that genotypes of this species performed similarly in ambient and high [CO2]. To circumvent challenges of whole genome association analyses in high diversity populations, we applied a two-step process involving, 1) whole-genome bulked segregant analyses (BSA) to identify candidate regions of the genome based on population extremes, followed by 2) genotype:phenotype association of SNPs in candidate regions. We identified 3641 and 3873 significant associations at a recommended FDR of 1.0X10-5, which was narrowed to 110 and 122 unique candidate genes for d13C and diameter respectively. In both cases this included a number of genes with functions related to environmental stress. To confirm associations we are in the process of screening SNP diversity from 2MB of the genome in each genotype of the association population via target enrichment sequencing, which will be validated firstly in glasshouse and then field collected trait data.

Ecological genetics and adaptation in European yew (Taxus baccata L.)

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Studies on how forest tree species adapt to major environmental drivers have acquired great relevance in the last years, fostered by increasing societal demands to produce sound forest management and conservation plans in a context of climate change. Some species, such as the European yew (Taxus baccata), due to their fragmented (albeit large) distribution and often low effective population sizes may be more severely affected by climate change than other forest trees. Here we provide an overview of our research on population genetics and adaptation in this iconic conifer. We first describe the multi-layered population genetic structure revealed by microsatellites at different spatial scales, from within-stand to rangewide. Second, we present quantitative genetics evaluations on a wide range of phenotypic data, including multi-year measurements of growth, phenology, and taxane (including taxol) content, from a clonal common garden planted in Central Spain. Phenotypic data allowed us to identify temperature as a main adaptive driver for European yew. Third, we present our ongoing work on molecular adaptation to climate based on 1,210 candidate genes (25,726 SNPs) obtained from an exome capture experiment covering an environmental gradient in Europe. First results based on new-generation outlier detection and environmental association approaches identified a methionine synthase, involved in responses to stress, as a target of selection in this species. Finally, we provide insights and perspectives for future research work on European yew molecular adaptation and its relevance for the species' management and conservation.

Signatures of local adaptation in candidate genes of oaks (*Quercus* spp.) in respect to present and future climatic conditions

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Testing whether and how populations are adapted to their local environment and predicting their response to future habitat alterations is of key importance in the face of climate change. A powerful approach to investigate such processes is landscape genomics, which identifies genes and environmental factors involved in local adaptation. We investigated whether the three common and ecologically divergent oak species in Switzerland (Quercus petraea, Q. pubescens, Q. robur) are

adapted to their present and future environmental conditions. In a pooled amplicon sequencing approach of 94 genes in 71 oak populations, we identified over 3P0 single nucleotide polymorphisms (SNPs) and tested if they show an association with abiotic factors related to local topography, historical climate, and soil characteristics. These environmental association analyses were run on all populations and on populations of each species separately. In each analysis, we found around 20% of the SNPs and 80% of the genes associated to at least one environmental factor. In the overall analysis, the most frequently associated environmental factors were those best describing the habitats of the different species. In the species-specific analyses, associated environmental factors and SNPs greatly differed among species. However, we identified one SNP and seven genes that were associated to the same environmental factor in all three species. We finally used allele frequency distributions of candidate SNPs along environmental gradients to predict the risk of non-adaptedness (RONA). RONA represents the change in allele frequency of populations that is theoretically required to be adapted to simulated climatic conditions in the future. Our results show that RONA is considerable for some populations and species. For example, in order to be adapted to the predicted warmer climate, populations of Q. robur need a change in mean allele frequency of 30% within 140 years in SNPs associated to temperature. However, RONA strongly differs among species and environmental factors and is therefore hard to predict for multifactorial environmental change. Given the long generation time of oaks, some of the required allele frequency changes might not be realistic for the populations to achieve on the basis of standing genetic variation. We conclude that some oak stands will require gene flow or plantations to introduce beneficial alleles or better adapted seed material (including different oak species) from habitats that currently match future climatic conditions.

Recombination rate variation, hitchhiking, and central-peripheral structure shape deleterious load in black cottonwood (*Populus trichocarpa*)

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Deleterious alleles that affect fitness are expected to be purged by purifying selection or maintained at low frequency by drift. However, many additional evolutionary forces may shape the pattern of deleterious mutations across the genome and among populations, including positive selection, hitchhiking, recombination, and demographic history. We used exome capture data to estimate the genome-wide distribution of deleterious alleles across natural populations of Populus trichocarpa. While deleterious alleles were on average present at low frequency, suggesting purifying selection, they were preferentially enriched both within genomic regions of low-recombination and in regions showing evidence of recent positive selection. In addition, the demographic history of poplar appeared to play a role in the distribution of deleterious alleles among populations, with peripheral populations having higher rates of deleterious homo- and heterozygosity. This suggests that marginal populations bear more deleterious mutations due in part to less efficient selection arising from smaller effective population sizes, and possibly also due to recent bottlenecks associated with postglacial recolonization. Finally, correlations between deleterious homozygosity and plant growth suggest a significant effect of deleterious load on fitness. Our results suggest that both genomic context and historical demography play a role in shaping the distribution of deleterious alleles across the genome and range of P. trichocarpa.

KEY NOTE: Matthew Rockman

Maternal and zygotic genetic effects in life-history evolution

Matthew Rockman
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Early stages of organismal development are subject to unique selective pressures that differ from those experienced by mature individuals. Early stages are also disproportionately dependent on maternally provisioned material. I will describe an experimental genetic model for the interplay of maternal and zygotic genetic effects on phenotypic variation and evolution. Females in our study species make either many small offspring or few large offspring, and the offspring develop through different morphologies and dispersal modes to yield indistinguishable adults. These developmental differences are highly heritable via both maternal and zygotic effects, with implications for how selection shapes the distribution of phenotypes in populations.

Polygenic adaptation in a hierarchical population structure

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Local adaptation is generally studied at a large geographic scale, i.e. among populations. However, within-population environmental heterogeneity can be large and there is increasing empirical evidence of adaptation at microgeographic scale (i.e. within dispersal neighborhood [1,2,3]). A recent review [4] advocates more studies on the interplay of evolutionary drivers at a wider range of scales.We used the NEMO simulation framework in a hierarchical island metapopulation model to investigate the evolutionary response to hierarchical patterns of selection both within and among populations. By varying strength of selection, demographic parameters and the genetic architecture of traits, we investigated polygenic adaptation patterns and processes at microgeographic and population scales by using generalized Q-statistics and Fq-statistics for hierarchical population structures.Our results show that microgeographic adaptation can be expected under a broad range of parameter values including relatively low selection intensity. Patterns of differentiation at QTLs (Fq-statistics) at all scales are primarily shaped by the pattern of environmental heterogeneity, the intensity of selection and the number of QTLs determining the trait under selection. We also show interactions across spatial scales: e.g. patterns of within-population heterogeneity have an impact on patch occupancy and differentiation between populations. Furthermore, the discrepancy between Q-statistics and Fq-statistics due to covariance among QTLs depends on the interaction between the differentiation patterns at different hierarchical levels of the population structure. These results will be discussed from the point of view of the expected genome-wide distribution of marks of selection, particularly those that can be detected as changes of QTL-allele frequencies.

Evidence for local adaptation and potential maladaptation to climate change in Fagus sylvatica: Genome-environment and phenotype-environment associations at regional scale

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The evolutionary potential of long-lived species, such as forest trees, is fundamental for their local persistence under climate change (CC). Genome-environment and phenotype-environment association analyses (GEA /PEA) can reveal whether populations in heterogeneous environments at regional scale are under differential selection pressure. This would result in populations with potential pre-adaptation to CC within this area. In addition, climate projections can indicate the risk of maladaptation to CC.In 79 natural Fagus sylvatica populations, neutral genetic structure was characterized by 12 nSSR-markers and potentially adaptive variation (144 SNPs in 52 candidate genes) was associated to 87 environmental predictors using latent factor mixed models (LFMM; [1]), logistic regressions and isolation by spatial/environmental distance (IBD/IBE) tests [2]. Seedlings of

the same populations were grown in two common gardens at contrasting altitudes to estimate quantitative genetic differentiation (Qst) and phenotypic plasticity in growth and phenology traits as well as to analyse PEA patterns [3]. To predict the risk of maladaptation to CC, differences between present allele frequencies or plant phenotypic traits to those expected under projected climatic conditions were assessed (analogous to [4, 5]).nSSR-diversity revealed low genetic differentiation (overall Fst = 0.017) and relatedness up to 150 m inter-tree distance. However, large-scale spatial genetic structure and IBE were absent. In the GEA 16 SNPs in 10 genes were associated to environmental predictors and IBE (corrected for IBD) was confirmed. The GEA often reflected the described gene functions, including evidence for adaptation to water availability and temperature. Qst was higher for the phenology than growth traits (0.18–0.32 vs. 0–0.16) and was often much larger than Fst, suggesting that most traits are under selection. Within-population genetic variation was greater for growth than for phenology. In the common garden at the high elevation, phenology was delayed and plants grew less, suggesting a passive plastic reaction. The PEA analyses indicated adaptive divergence in phenology and growth with respect to temperature and water availability, but not to soil characteristics. Overall, climate models explained 19–42% of the seedling trait variation. Finally, potential maladaptation to climate change was found for different traits and populations.In our study system, genomic and quantitative genetic divergence, combined with a lack of large-scale neutral genetic patterns suggest that gene flow allows the spread of advantageous alleles in adaptive genes and traits. Therefore, adaptation processes are likely taking place in populations occupying heterogeneous environments, reducing their regional risk of maladaptation under CC.

Daily stem radial fluctuations in Eucalyptus reveal the dynamic of the genetic determinants of trees response to environment

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In trees, daily stem radial fluctuation (DSRF) constitutes a remarkable dynamic trait in response to environmental variations. This circadian rhythm integrates two major processes: i/ the variation in water content in stem tissues in relation with water transport in the stem, determined by transpiration and water absorption, and ii/ cell division in the cambium and subsequent cell expansion (secondary xylem and phloem). DSRF can be measured at a very fine temporal scale using automatic dendrometers placed on the surface of the trunk. While it has been largely studied by ecophysiologists, the genetic basis of DSRF and its interplay with the environment remain largely unknown.The objective of this study was to characterize the genetic architecture of the DSRF in Eucalyptus, to learn about the dynamics of genotype-by-environment interaction (G×E). To this end, we analyzed two years of sub-hourly data collected on 220 full-sibs of E. urophylla x E. grandis grown in the Republic of Congo, i.e. about 200,000 data points per tree. Two components of the circadian cycle were studied: the daily amplitude of radial shrinkage (DA), and the difference between successive daily maximum trunk radius values (ΔRm). These two variables in relation with environmental factors (temperature, air vapor pressure deficit, soil water content, global radiation) enabled us to study the QTL×E interaction.At the phenotypic level, DA and ΔRm were on average not correlated during the studied period. They showed clear differences in environmental plasticity: DA being positively correlated with several atmospheric variables (vapor pressure deficit, radiation and temperature) at the daily scale. Conversely, ΔRm showed low correlations with environmental variables at this time scale, while it was associated with soil water content over a longer integration time (week). These two traits presented a very different genetic architecture (few coincidences between QTLs) suggesting that different genes are indeed involved in DA and ΔRm variation. Moreover, the study of the genetic architecture of both traits over two years revealed a temporal instability of the genetic control for DA and ΔRm. For DA, this instability was clearly related to seasonal variations, and well-illustrated by two QTL regions on the linkage group 3 of the E. urophylla. Interestingly, these QTLs colocalized with two major QTLs involved in the genetic control of mature wood properties. These results reinforce the hypothesis that tree response to environmental variation at the juvenile stage could be a key driver of many properties at an older developmental stage.

Validating climate-adaptation candidate genes and translating variation into assisted gene flow strategies

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Population genomic approaches can characterize local adaptation to climate and inform assisted gene flow strategies more quickly than provenance trials by revealing geographic variation in climate-related genes and identifying climatic drivers of that variation. Analytical challenges include a high rate of false positives for climate adaptation if population structure is not controlled in analyses, false negatives if population structure and demographic history parallel spatial patterns of variation, and the high threshold for statistical significance necessary with large numbers of markers (Lotterhos and Whitlock 2014, 2015). Once climate-adaptation genes and climatic drivers of local adaptation are validated, the next challenge is how to translate knowledge of that variation into guidelines for reforestation in new climates. Here we report on various approaches we are using to validate candidate genes for climate adaptation and translate patterns of adaptation into recommendations for assisted gene flow (Aitken and Whitlock 2014) in the AdapTree Project.We re-sequenced ~23,000 genes in ~600 trees from over 280 populations of lodgepole pine (Pinus contorta) and 250 populations of 'interior' spruce (Picea glauca, P. engelmannii and their hybrids). Genetic-environment associations (GEA) and genotype-phenotype associations (GPA) with climate-related traits were used to identify single nucleotide polymorphisms (SNPs) and candidate genes involved in local adaptation. Top candidates in each species were identified as those genes with exceptional numbers of SNPs in the 99th percentile for GEA and GPA tests adjusted for population structure. We also tested orthologs of top candidates identified in one species in the other, using raw rather than population-adjusted associations. This comparative approach identified substantial overlap between these long-diverged (~140MY) taxa, and suggested single species analyses resulted in over-correction for population structure in interior spruce. The primary climatic drivers of local adaptation identified by GEA were related to low temperatures rather than precipitation for both species, and are consistent with phenotypic variation (Liepe et al. 2016).Climate-adaptation candidate SNPs from GEA and GPA were included on 50K SNP arrays for each species. These arrays have been used to validate SNPs by genotyping several thousand trees per species from: 1) additional seedling common garden experiments and environments; 2) multiple sites and populations in a provenance trial; and 3) selectively bred orchard seedlots from regional breeding programs. Validated SNPs will be used in regression tree and climatic niche models to cluster similarly adapted natural stand and breeding populations for genetic resource management, and to match adaptive profiles to current and future climates for assisted gene flow.

SESSION 3

KEY NOTE: Rishikesh P. Bhalerao

Molecular basis of adaptation to seasonal changes in boreal trees

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Trees growing in temperate and boreal forest need to modulate their patterns of growth in order to survive the extreme fluctuations in temperature that accompany the change of seasons. Therefore these trees undergo growth cessation and establish dormancy prior to the advent of winter when temperatures can go down to -50℃. The cessation of growth and establishment of dormancy is controlled by photoperiod. Whereas release of dormancy and subsequent bud flush in the spring are temperature regulated. In contrast to photoperiodic control of growth cessation, the molecular basis of bud dormancy establishment, its release and bud flush is poorly understood. We have identified evolutionarily conserved components of Polycomb and plant hormone ABA as key components of SD mediated bud dormancy establishment. I will discuss the how polycomb and ABA mediate in photoperiodic control of bud dormancy establishment. I will describe our recent results that provide insight into the genetic framework underlying temperature controlled break of dormancy and subsequent bud flush.

Adaptive Variation in Lodgepole and Jack Pine Population Responses to Mountain Pine Beetle Fungal Associates and Abiotic Stresses

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Mountain pine beetle (MPB) is an historic component of pine forests in the central and southern interior of British Columbia. However, in recent years MPB populations have expanded north- and eastward, threatening pine forests naive to attack from this pest. This study investigates adaptive variation in lodgepole pine, the ancestral host, and jack pine, a naive host, across east-west gradients in Canadian pine populations for traits that influence host quality to MPB. Characterizing the susceptibility of naive pine populations is important for predicting the impact MPB will have as it expands beyond its historic range. Lodgepole pine is distributed throughout the western North America, moving eastward we transition to jack pine, whose distribution range extends from Alberta to the Atlantic provinces. Using population genomics, we have redefined the large hybrid zone that occurs between these two species in Alberta and discovered introgression and patterns of diversity that suggest genetic variation is linked to variation in environmental factors along the east-west gradient. We hypothesize that genetic variation along this gradient in lodgepole and jack pine also correspond to a gradient in susceptibility to MPB fungal associates (Grosmannia clavigera), and that traits affecting tree defence capacity, such as drought tolerance, differ across these same gradients. To test these hypotheses we conducted a large-scale phenotypic screening of seedlings from 18 pine provenances from across Canada for response to G. clavigera infection and to drought. We measured lesion length at 1 and 2 weeks after infection with G. clavigera and photosynthetic capacity at 1, 3, 5 and 11 weeks of drought conditions in 756 and 816 trees, respectively, from our 18 populations. We observed wide variation between our populations in phenotypic response to our biotic and abiotic treatments. We are currently preparing to conduct a genome-wide association study to identify genomic regions that underlie this variation. The results of this work contribute to our understanding of how genetic variation in pine contributes to differences in host quality and, by combining these results with our population genetic work, will identify whether population genomics can identify spatial patterns of differential pine susceptibility to mountain pine beetle in novel habitats.

The nature of the progression of drought stress drives differential metabolomic responses in Populus deltoïdes

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Use of woody crops for Quad-level (1015 BTU) energy production will require use of marginal agricultural lands, where the occurrence of water stress will be recurrent, especially given the predictions of increased frequency and severity of droughts associated with the predicted global climate change. Our previous research demonstrated that Populus has the capacity to increase dehydration tolerance by lowering the osmotic potential via osmotic adjustment, the active accumulation of solutes under stress, which allows turgor and growth maintenance under mild to moderate stress and facilitates growth recovery after stress relief. Despite the large number of drought stress studies that have been published, few studies have contrasted the degree and nature of solute accumulation if the nature of drought stress progression is varied (e.g., cyclic vs acute, short-term vs long term, moderate vs severe stress).

We conducted a drought stress study on Populus deltoides in a greenhouse and determined the metabolomic profiles of leaves from plants subjected to mild (-0.5 MPa predawn leaf water potential) cyclic drought vs severe (-1.30 MPa) in contrast to well-watered controls (-0.1 MPa) after 2 or 4 drought cycles, and in contrast to plants subjected to acute drought, where plants were not rewatered and were allowed to dessicate for up to 8 days. Leaves were rapidly sampled, fast frozen on dry ice, ground with liquid N and then twice extracted with aqueous ethanol (80%). Dried aliquots of extracts were analyzed for metabolites by gas chromatography-mass spectrometry (GCMS) with electron impact ionization (70 eV) following trimethylsilylation.

The nature of drought onset (cyclic vs acute), frequency of drought (number of cycles), and the severity of drought (mild vs severe), all interact to dictate the degree of osmotic adjustment and the nature of the organic solutes that accumulate. Acute onset of prolonged, severe drought induced the greatest osmotic adjustment after withholding water for 7 days (1.44x) with the greatest accumulations

in the large, complex higher-order salicylate conjugates. Organic solute accumulation under cyclic stress relative to well-watered controls was moderate (1.33x) and was largely constituted by soluble sugars, organic acids, and amino acids. The metabolite responses are discussed in the context of stress acclimation versus metabolic perturbation.

Genome-wide transcriptome and miRNA profiling of epigenetic memory formation in somatic embryos of Norway spruce in response to temperature conditioning

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Epigenetic memory in Norway spruce affects vitally important adaptive traits such as the timing of bud burst and bud set. The temperature conditions that prevail during early seed formation impact on epigenetic processes resulting in the formation of epitypes, which determine the seasonal timing of bud burst and set throughout later developmental cycles and seasons. Epigenetic memory formation involves multiple epigenetic regulators and small non-coding RNAs. Components of epigenetic regulators include writers, eraser and readers of epigenetic marks on DNA and chromatin. MicroRNAs (miRNAs), a class of small noncoding RNA molecules have recently drawn attention for their prominent role in development, phase change and in their role in mediating response to environmental conditions. Understanding the complex genetic pathways and regulatory mechanisms operative during epigenetic memory formation was feasible by combining data of genome-wide transcriptome and profiling small RNAs produced by different conditioning temperatures.We prepared 18 small RNA and 18 mRNA transcriptomic libraries from embryogenic tissues of two individuals at three stages of maturation grown in vitro under three epitype-inducing temperatures (18, 23 and 28℃). These libraries were sequenced with the PGM™ (Ion Torrent™) system and were analyzed using CLC genomic workbench software.The epitype-inducing conditions during somatic embryogenesis were accompanied by marked transcriptome changes for a large number of gene models related to the epigenetic machinery. Out of 735 putative orthologs of epigenetic regulators, 329 were affected by the epitype-inducing temperatures and differentially expressed. Remarkably, we observed enrichment in the majority of differentially expressed genes for epigenetic regulators related to DNA and histone methylation, sRNA biosynthesis and action, and putative thermo-sensing and signaling genes. Extensive characterization of miRNAs in Norway spruce during different stages of embryogenesis under different temperature treatments allowed the identification of 1115 highly expressed miRNAs, including 522 conserved and 593 novel miRNAs. For most miRNAs, we confirmed precursor molecules and defined their cognate targets. We demonstrate that Norway spruce has a variety of miRNAs with distinct epitype inducing temperature dependent expression patterns, and that these miRNAs target an asymmetrically higher number of spruce genes with broad functions, including those involved in epigenetic regulation. Overall, 124 miRNAs targeting 203 differentially expressed epigenetic regulators were defined, l indicating an expansion of the epigenetic machinery relative to angiosperms, and we suggest that this expansion reflect a finely tuned mechanisms involved in development and in epigenetic memory formation.

Endogenous rhythmic growth, a trait suitable for the study of interplays between multitrophic interactions and tree development

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As perennials, trees undergo phases of meristem activity ceases that control tree architecture and their ability to survive through unfavorable seasons. The episodic growth is linked to variations in allocation of the below- and aboveground plant resources. This affects the numerous multitrophic associations typical of trees, which feedbacks on plant growth and resource economy. Some major forest trees display an endogenous growth rhythm, and related pulses of variation in allocation of resources have been detected. As this trait makes it possible to separate growth into defined phases, it offers an opportunity to disentangle the complex regulation of growth and multitrophic interactions. We present the experimental platform “TrophinOak” [1] that uses microcuttings of DF159, a clone of Quercus robur. Q. robur displays an endogenous rhythmic growth characterized by alternating shoot and root growth flushes. We selected beneficial or detrimental above- and belowground partners including animals, fungi and bacteria, to synthesize multitrophic interactions on DF159. At distinct phases of the oak growth, fluctuations of carbon and nitrogen allocation were monitored by stable

isotope pulse labeling, and plant gene expression was analyzed by RNA-Seq in reference to a contig library specific of DF159 [1, 2].We found highest variations in gene expression in leaves and roots entering into growth cessation at the end of shoot and root flushing, respectively. Plants inoculated with the ectomycorrhizal fungus Piloderma croceum displayed enhanced growth and increased resources without modifying the rhythmic growth. However in this inoculation treatment, the differential gene expression in leaves and roots entering into growth cessation dropped, especially in roots. This enabled us to detect core genes ruling endogenous rhythmic growth, several of which correspond to internal clock processes [3].Comparing interactions with seven types of biotic partners, we found only two patterns of differentially expressed contigs. Patterns 1 corresponds to increased levels of DEC upon shoot flush in both root and shoot tissues, while in pattern 2 the increased level is found during root growth flush. Noteworthy these patterns depend neither on the beneficial or detrimental nature of the partners, nor on whether they target leaves or roots. We now aim at identifying genes common and specific of the different types of biotic partners [1].(1) Herrmann et al. (in press) Perspectives in Plant Ecology Evolution and Systematics(2) Tarkka et al. (2013) New Phytologist 199, 529-540(3) Herrmann et al. (2015) Journal of Experimental. Botany 66, 7113-7127

Epigenomics and tree phenotypic plasticity in response to water deficit

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Global climate changes in progress will impact tree productivity in the northern hemisphere. The understanding of physiological as well as genetic/molecular processes controlling plants response to abiotic stress will help to improve plant breeding. Recently, epigenetic mechanisms such as DNA methylation have been shown to participate to the control of plant development and their adaptation to environment through modifications of chromatin compaction and gene expression profiles. Phenotypic plasticity defines as the different phenotypes for a given genotype in distinct environments is a key process for plant to adapt to their changing environment. This is particularly relevant for perennial plants such as trees that are exposed to repeated fluctuations of their living conditions. In this context, drought is a significant threat to forest health and agro-ecosystem productivity. With the availability of its genome and its important natural genetic and phenotypic variations, Populus became a model tree.The aim of our project is to develop an integrative approach to identify ecophysiological and molecular bases, particularly DNA methylation, involved in phenotypic plasticity. Contrary to animals, plants exhibit a continuous development and their germline arise from very specific somatic tissue: the shoot apical meristem. We focus our effort to the study the role of DNA methylation in shoot apical meristem using various epigenomic approaches (MeDIP-SEQ, MeDIP-CHIP, WGBS) in parallel to transcriptomics and phenotyping. Our experimental designs include collection of genotypes and are established in controlled conditions such as in greenhouse and nursery as well as in field plantations. We have also characterized the first hypomethylated tree (DDM1-RNAi poplars) in different environments.Altogether, our results demonstrate the role of DNA methylation in memorizing environmental influence and identify the corresponding genes network in relation to phenotypic plasticity.

KEY NOTE: Joy Bergelson

Diffuse interactions shape the dynamics of a plant pathogen interaction

Joy Bergelson
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Theories of host pathogen interactions explain the maintenance of resistance polymorphisms in terms of frequency dependent selection acting between an obligately associated host and pathogen. However, many host-pathogen interactions are not obligate: pathogens often infect multiple host species and hosts harbor complex microbial communities. In this talk, I will tease apart the ecological interactions underlying an ancient balanced polymorphism that we have identified in Arabidopsis thaliana in nature. We find that this ancient balanced polymorphism at the R gene, Rps5, persists amidst a web of complex interactions involving multiple host species, multiple bacterial species and multiple effectors segregating among strains of single pathogen species. These results challenge us to understand how selection acts on plant resistance, and how pathogens adapt to their numerous secondary hosts. I will assess how P. syringae adapts to one of its secondary hosts, A. thaliana, through an analysis of whole genome sequences and associated experiments.

Discovering genes involved in ecological speciation in European white oaks using an RNAseq approach

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The European white oaks are mainly represented by two species: sessile (Quercus petraea Matt. Liebl.) and pedunculate (Quercus robur L.) oaks [1]. The distribution of these two sympatric species, is largely imposed by environmental factors such as light, soil composition and humidity [2]. Sessile oak is generally found on deep, well drained and rather acidic soil, while pedunculate oak favors deep and fertile bottomland with sometime large level of hydromorphia. It is more tolerant to root hypoxia, whereas sessile oak is more drought tolerant [3]. This species complex constitutes therefore a powerful system to determine the role of gene expression in ecological speciation. While the role of gene expression in ecological speciation is receiving a growing interest in animals, it still remains in its infancy in forest trees. To fill this gap, we exposed seedlings of these two oak species to a cycle of waterlogging followed by a moderate drought after a recovery period. White roots were harvested on the two species along the whole cycle. An additional sampling of the immersed portion of the stem (i.e containing hypertrophied lenticels) was also performed after the seedlings received the waterlogging treatment. Gene expression for the different time points of the kinetics (5 in total) and tissues was analyzed using an RNAseq approach. Genes showing differential expression between the two species were identified using the Deseq and the EdgeR software. Overall, 1,997 and 107 genes were found to be over-expressed in pedunculate oak and in sessile oak during waterlogging and drought stress, respectively. Genes regulated by waterlogging and drought stress were validated by qPCR. Our results provide new insights on the role of gene expression in shaping adaptive phenotypes in these two oak species. For instance, we found that the suberin pathway is an important component of waterlogging adaptation in pedunculate oak by favoring O2 diffusion to the root tip.

Tissue-specific transcriptome profiles underlying genotype- and drought-related variation in phenotypic traits in Populus nigra

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Considering the increasing demand for both, food production and production of bioenergy feedstock, energy crop production systems should preferentially utilise perennial crops grown on marginal sites. Climate change, especially drought events, put biomass productivity at an increasing risk. Therefore, it is pivotal to understand the underlying physiological and molecular mechanisms of drought stress responses of bioenergy crops, and to investigate genotypic variation of such drought stress responses. Here, we report on a study of drought stress adaptation in Populus nigra. Our hypothesis is that genotypes from environments differing in precipitation and mean annual temperature harbour different physiological and molecular mechanisms to cope with limited water availability, which can be exploited to produce improved germplasm for sustained biomass production.To address this hypothesis plants of three genotypes of Populus nigra originating from habitats characterized by increasing water limitation were exposed to well-watered conditions or moderate drought for five weeks in a greenhouse experiment.We found constitutive differences in biomass productivity among genotypes, and a significant reduction of biomass accumulation under drought. Height and diameter growth rates differed among genotypes and were reduced early after the onset the drought treatment. Drought provoked an increase of intrinsic water use efficiency, with some genotype effects. The genotypes differed constitutively in wood anatomical traits, and some traits were affected by drought.RNA-seq transcriptome profiles obtained from plants sampled at the end of the experiment revealed a strong drought-effect in mature leaves and fine roots, while the transcriptome of the developing xylem was more resilient. In addition, very strong genotype effects were detected, while only a small number of genes showed genotype-×-treatment-interactions. By gene correlation network analyses, we identified genes and gene clusters linked to drought-related and genotypic variation in growth and biomass traits.AcknowledgementsThe research reported here was conducted in WATBIO (Development of improved perennial non-food biomass and bioproduct crops for water-stressed environments) which is a collaborative research project funded from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 311929.

Low temperatures induce secondary cell wall remodeling in Eucalyptus through a complex network of transcription factors

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The fast growing eucalypts are by far the most widely planted broad-leaved trees around the world. They provide one of the best sources of lignocellulosic biomass for pulping and are emerging as a valuable alternatives to fossil fuels. Recently, Eucalyptus was officially approved for forest plantation in France, but frost still represents the main limitation for the expansion of this non-dormant tree. Besides the design of breeding strategies aimed at selecting frost resistant clones, it is important to understand the effects of low temperatures on wood production and quality which directly impact industrial end-uses.To evaluate cold effects specifically on wood tissues formation, we performed an integrated approach combining xylem histology, biochemical characterization and gene expression profiling. We performed a long-term cold acclimation (4℃ during 7 weeks) in controlled condition using commercial Eucalyptus gundal clones (provided by FCBA, France). Secondary stems were harvested at different time points (0, 2, 15 and 49 days after cold exposure). Klason, thioacidolysis and analytical pyrolysis were performed to analyze lignin content and composition. Histochemistry analyses coupled with automatic measurements of xylem cell parameters (ImageJ software ©) were performed on LR White (SIGMA) resin embedded xylem sections. Expression profiles of more than 100 genes related to secondary cell wall biosynthesis and regulation were assessed in each sample using high throughput Real Time qPCR (Fluidigm Biomark®).Low temperatures had strong effects on xylem cells. Secondary cell wall thickness was significantly increased in fibers, parenchyma cells and vessels. By comparing transverse stem sections of Eucalyptus grown at 25℃ and 4℃, we showed that the lignification

patterns of the more recently differentiated xylem cells were altered. Low temperatures triggered deposition of lignin in cells located immediately close to the cambium and a significant increase of lignin content. Expression profiles clearly showed an induction of genes related to lignin, cellulose and hemicelluloses biosyntheses and of several transcription factors recently identified (Soler et al, 2015; Hussey et al, 2015). These results were confirmed using adult trees xylem samples harvested in plantation conditions.Finally, gene co-expression network analyses pointed out new regulators at the crosstalk of abiotic stress responses and secondary cell wall formation. These genes are under functional characterization in transgenic poplars and Eucalyptus hairy roots (Plasencia et al, 2015).

Evolution of a resistance mechanism in conifers

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Defense strategies diversify, converge, or are conserved in plants, however, evolutionary patterns of defense are mostly unknown in trees. Recently, a resistance mechanism against spruce budworm (Choristoneura fumiferana, SBW), the most destructive forest insect in eastern North America, was identified in white spruce (Picea glauca). In this mechanism, the enzyme PgβGLU-1 catalyzes the cleavage of acetophenone sugar conjugates to release the aglycons that are toxic for SBW. We conducted two studies to characterize micro and macro scale patterns of evolution of this novel resistance mechanism.At the microevolutionary level, temporal and geographic variation in resistance traits was investigated in white spruce. During June, aglycon aceptophenone concentrations rise in current-year foliage, coinciding with the peak of SBW damage in white spruce. A positive relation was also observed between the aglycon acetophenone concentration in foliage and the duration of development in SBW females, indicating a negative impact of foliar acetophenone levels on insect fitness. At the geographic level, traits associated with resistance were not fixed within the natural population of white spruce. Resistant trees abounded in areas with the highest SBW historical damage or contemporary conditions favorable to SBW.At the macroevolutionary level, Pgβglu-1 gene sequence, structure, and expression as well as foliar acetophenone concentrations were compared between five spruce species and one fir, all affected by SBW (P. glauca, P. mariana, P. rubens, P. abies, Abies balsamea). Spruces and the fir species have highly similar Pgβglu-1 gene sequences (> 95%, 3485 bp). In contrast, Pgβglu-1 gene expression and acetophenone concentrations varied widely across species. P. glauca and P. abies had similar wide ranging variation in expression, and the three other species had low expression. All of the spruce species have at least one glucosylated-acetophenone, but A. balsamea does not produce any acetophenones.Together these findings show that SBW selects for resistant trees in the white spruce population. On the one hand, temporal and geographic variations in resistance traits support the crucial role of this mechanism in species survival against its most damaging natural enemy. On the other hand, results from the interspecific analysis indicate that the gene and traits associated to the resistance mechanism did not evolve solely in white spruce, but may have been selected in this species for its survival. This new understanding of SBW-spruce interactions and co-evolution will support applications in resistance breeding and planning for integrated pest management.

Responses of an evolutionarily co-evolved and a naïve pine host in the face of mountain pine beetle range expansion

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The current epidemic of mountain pine beetle (Dendroctonus ponderosae) has impacted more than 28 million hectares of pine forests in western North America. Lodgepole pine (Pinus contorta var. latifolia), with a range overlapping that of mountain pine beetle, has been the main species affected by the outbreak. From its historic range in British Columbia, this devastating bark beetle has spread across the Rocky Mountains into northern Alberta. In this novel habitat, lodgepole pine hybridizes with jack pine (Pinus banksiana), a sister species whose range extends across the boreal forest to Atlantic Canada. We have demonstrated that mountain pine beetle has undergone host range expansion to

pure jack pine in north central and north eastern Alberta. We are testing the hypotheses that (1) host quality differs between lodgepole and jack pine and (2) that water limitation affects these responses. Both lodgepole and jack pine exhibit conservative water use strategies in response to water deficit. Lesion development following inoculation with the mountain pine beetle pathogenic fungal associate Grosmannia clavigera was slower in jack pine than lodgepole pine, with water deficit delaying lesion development in both species. G. clavigera inoculation significantly increased bark levels of jasmonic acid in both species. While water deficit did not significantly affect jasmonic levels either in the presence or the absence of the pathogen, abscisic acid levels in bark increased significantly in response to water deficit, raising the possibility that abscisic acid signaling contributes to modulated defense responses under conditions of water deficit. Bark monoterpene profiles of lodgepole pine show greater responsiveness to G. clavigera inoculation than those of jack pine. Constitutive levels of several monoterpenes were enhanced under water deficit conditions, while induction of monoterpenes was largely attenuated under water stress. Microarray analyses revealed that thousands of genes are invoked in the response of these pine species to G. clavigera infection, that there are substantial differences in responses of lodgepole and jack pine, and that water limitation alters this transcriptional programme. As a first step to linking genotype with phenotype, we have identified a set of putative defense-associated genes whose expression levels change in response to G. clavigera, and also show signatures of selection. Taken together, these data suggest that molecular-level differences in responses of lodgepole and jack pine to the mountain pine beetle fungal associate G. clavigera contribute to differences in host quality between these species, and that these responses are compromised by water deficit conditions.

SESSION 4

KEY NOTE: Myriam Heuertz

Hybridization and the evolution of tropical tree species complexes

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Tropical rainforest tree genera often comprise high numbers of species, and closely related species commonly occur in sympatry. There is little information on the proximal genetic mechanisms to explain these patterns of species coexistence. Theoretical models predict that inter-specific hybridization could represent a key factor in the maintenance of highly diverse communities, notably by retarding the (local) extinction of rare species and by allowing the sharing of beneficial genetic variants across species borders. Hybridization could be an especially relevant evolutionary process under climate change, where changes in relative species abundance, distribution ranges and/or phenology can affect interspecific gene flow, and where the sharing of adaptive genetic variation could allow a faster response to environmental change.We set out to empirically test the importance of hybridization in closely related tropical tree taxa, using as models the genus Symphonia (Clusiaceae) in Madagascar and the Bertholletia clade (Lecythidaceae) in French Guiana. We sampled sympatric populations of each complex and characterized samples at genetic markers (cpDNA, SSRs, SNPs or RAD-seq). Symphonia holds ca. 20 species endemic to Madagascar. However, taxonomic species boundaries are often poorly defined, and discriminant morphological characters for species delimitation are often unknown or unavailable on many herbarium vouchers. We thus applied genetic clustering approaches on “blind samples” (i.e., without using morphology) for taxon delimitation, we constructed a phylogenetic hypothesis and characterized patterns of admixture between taxa. In the Bertholletia clade, a pilot RAD-seq experiment allowed us to assess genomic patterns of allele sharing, a first step in characterizing the genomic dimension of introgression. In both complexes, plastid DNA sequences

suggested evidence for plastid DNA sharing between several taxa. Our ongoing work provides evidence for variable levels of allele sharing and genomic admixture between sympatric species in two tropical tree species complexes from different continents, suggesting that inter-specific hybridization took place in their evolution.

Demographic and ecological factors operating at different temporal and spatial scales shaped the evolution of Mexican firs (Abies, Pinaceae)

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Divergence of taxa with life-history traits that favour large effective population sizes and retention of ancestral polymorphisms (i.e. outcrossing mating systems, high longevity, predominant position in the ecosystem, and wind pollination) often coincide with models of evolutionary stasis, decreased extinction and niche conservatism. Diversification rates in these taxa may however increase when they evolve in heterogeneous environments that prompt temporary fragmentation, isolation by distance, and disruptive selection, like in the mountain systems of subtropical countries. We surveyed this possibility by analyzing the genetic variation among and within fir (Abies) species in Mexico at various geographic scales. Fir forests in this country are distributed in large and scattered mountain populations that grow along a latitude gradient between the tropical highlands in the south (elevations higher than 3,000 m above sea level (masl)) and the subtropical ranges in the north (~1,500 masl). Further palinological and geological evidence has showed that these populations experienced recurrent expansions, contractions and fragmentations during the many magmatic events that have occurred in Mexico since the Pliocene, and throughout the glacial cycles of the Pleistocene. We first reconstructed climate niche models and phylogenetic relationships for the Mexican Abies. Using 12 single copy nuclear genes, we obtained a phylogeny that was in agreement with but had more resolution than previous attempts with chloroplast DNA. It particularly showed a significant correspondence between the main clades and some of the mountain ranges where the Mexican Abies currently grow. While A. concolor and A. durangensis var. coahuilensis had the most divergent climate niches, all species conformed fairly well to a model of niche conservatism when examined across the phylogeny. Several tests for selection suggested that adaptation may have also been involved in species divergence. Two genes were highlighted on these tests and through simulations used to survey the divergence of a species pair (A. flincki and A. religiosa) from central Mexico, particularly in the clade leading to A. flincki. A landscape survey performed at the local scale finally revealed that strong selective forces may also be operating at such short distances. Divergence of Mexican firs may thus resemble a continuum process leaded by genetic drift and adaptation in the presence of gene flow, which have occurred across various temporal and spatial scales and that blur most traditional taxonomic boundaries recognized so far.

Napoleonian legacy in Populus nigra populations

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Black poplar (Populus nigra), a major dioecious tree species in riparian ecosystems in Europe, is endangered by loss of habitats and gene flow from cultivated poplars. These included not only exotic species present in commercial plantations but also ornamental species widely planted known as cv. 'Italica' (Lombardy poplar). The latter origin is supposed to be Middle East, and was introduced in European landscape 250 years ago, and in France during the Napoleonian era. This cultivar belong to the P. nigra species and is known to be the male parent of several widely planted interspecific hybrid cultivars.A 12K Infinium beadchip array was developed to study P. nigra diversity in Western Europe (Faivre-Rampant et al. 2016). A total of 1150 P. nigra individuals from natural stands and progenies from controlled crosses were genotyped. The cv. 'Italica' and related ancient cultivars were also included. Structure of the populations from natural stands studied with a total of 7896 SNPs reflected the geographical distribution of the species in major river basins but a high level of admixture between populations remained. A non-negligible part of this admixture may be due to 'Italica' genetic

background as this cultivar and introgressed individuals formed a distinct genetic pool. The rate of introgression differed between populations, French populations being the mostly affected. Comparison with controlled crosses and ancient cultivars allowed to resolve pedigree of some introgressed individuals.We identified a set of alleles at SNP markers that are specific to 'Italica' which allow us to diagnose hybridization but also introgression in advanced generations. This work showed that the impact of the presence of P. nigra cv. 'Italica' in European landscapes on diversity of P. nigra populations is unexpectedly high.

Phylogenomics in tropical Fagaceae: an example from Castanopsis (D. Don) Spach

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Asia is one of the world's most diversified regions, in terms of environmental variation as well as in biodiversity values, mainly due to climatic oscillations during the quaternary, as well as to the geological changes that occurred from 30mya to present. However, a great part of this biodiversity remains unknown, and a phylogenetic framework is still lacking for many taxa in the region. The genus Castanopsis (D. Don) Spach (Fagaceae) comprise 110-134 species, which are restricted to tropical and subtropical eastern Asia (from Japan to Indonesia), some of them being very common in subtropical forests. About half of the species are found in China, and 30 are endemic, in a wide range of habitat types, where they are often keystone species. Despite they are sometimes locally abundant, several species are currently threatened and Red Listed.With the recent development of next-generation sequencing methods, it is now possible to study complete genomes to precisely delineate the history and diversity of taxa. However, Whole Genome Sequencing remains unaffordable for many non-model taxa. For these groups, highly repeated regions sequencing, including chloroplasts and nuclear ribosomal cistrons, has been showed to be an efficient method to reconstruct evolutionary history.Using N.G.S., one gigabase of genomic sequence was produced and assembled in complete chloroplasts and nearly complete cistrons for a subset of species in the genus and outgroups. We then used both nuclear and chloroplast data to performed phylogenetic analyses, molecular dating and biogeographical reconstruction to delineate the phylogeographic history, the diversification and adaptation patterns and processes of the genus.The close affinity between the genera Castanopsis and Castanea was confirmed, as well as the monophyly of the genus. Dating and biogeographic analyses indicated that the mid-Miocene climatic optimum likely drove the divergence between the two genera. We found that climatic and geological changes occurring in Asia during the Cenozoic were a major driving actor in the diversification of the genus, especially the uprising of the Himalaya mountain chain and eustatic sea level variations.We expect this study will serve as a cornerstone in improving our understanding of the origin and geographic distribution of genetic diversity in tropical trees, thereby aiding ongoing conservation efforts.

What do we mean by oak species and the oak phylogeny? Oak species coherence and parallel ecological diversification in the red and white oaks

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Oaks (Quercus: Fagaceae) hybridize readily. But evidence accruing over the past 20 years suggests that while there is certainly hybridization and introgression among oak species, gene flow amongspecies may be swamped by homogenizing gene flow among populations within species. I present data demonstrating oak species genetic coherence across wide geographic ranges in both the red and white oaks of North America, based on a combination of restriction-site associated DNA sequencing (RADseq), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) data. With particular focus on phylogenomic reconstruction of the evolution of the North American white oaks based on restriction-site associated DNA sequencing (RADseq) in comparison with whole-plastome sequencing, I show that the story of rampant interspecies gene flow suggested by the chloroplast does not dominate the phylogenetic signal in the nuclear genome. To the contrary, where the plastome shows a seeming panmixia among 12 white oak species, the RADseq data support clear and strongly supported species coherence and phylogenetic signal that is structured along morphological and biogeographic lines. I then turn to a comparative RADseq phylogeny of the red and white oaks, which have diversified particularly rapidly, producing ca. 250 species that range

from the Pacific coast to the Atlantic and from Canada to Central America. The phylogeny demonstrates parallel ecological and biogeographic diversification, as both clades have filled many of the same edaphic and climatic niches. Red oaks and white oaks may be found growing together throughout the continent, rather than separating along clear ecological or biogeographic gradients. This parallel history of species divergence likely explains much of diversity and ecological importance of oaks in the Americas.

KEY NOTE: Paul Manos

Mighty oaks from little acorns grow: phylogenomic data add new branches to the Quercus tree of life

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With over 430 species, the oak genus Quercus plays a major ecological role in a variety of ecosystems throughout the northern hemisphere. While oak species diversity and ecological function is well described, the impact of hybridization on evolution above the species level is not as clear. A rapidly developing molecular toolkit has advanced the systematics and biogeography of Quercus, increasing our understanding of the biology and relationships of species. A species-level phylogeny using restriction-site associated DNA sequencing (RADseq) confirms a basic geographic split between Nearctic and Palearctic oak clades, providing unprecedented levels of resolution. The RAD data strongly support eight lineages within the genus. In the Americas, combining phylogeny and fossil and modern distributions with other clade-specific data supports diversification scenarios, including two intercontinental disjunctions. The two largest clades in the genus, Quercus (white oaks) and Lobatae (red oaks), share strikingly similar biogeographic histories. The pattern suggests originations and deeper evolutionary splits at higher latitudes, followed by more recent parallel dispersals and diversifications south to Mexico and Central America. Comparing phylogenies to the results of data analyses using subsamples along multiple parameter axes uncovers evidence for hybridization between a few species from distinct clades. Localized hybridization based on morphology and geographic proximity explains some of these cases, but these analyses also point to more cryptic scenarios involving ancient introgressive hybridization among geographically disjunct and distantly related species. These results demonstrate that a combination of divergent and reticulate processes have promoted the diversification and spread of these ecologically and economically important tree species.

Waterlogged Oak Wood from across Europe Provides New Source for Tree Ancient DNA

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Understanding phylogeography and evolutionary responses of forest tree species to environmental changes is paramount in the face of the ongoing global warming. Long-lived species, including trees from all forest ecosystems, are of particular concern as their long generation times might limit their ability to adapt to changing environmental conditions. Diachronic studies using ancient populations can provide valuable complementary inferences on phylogeographic and evolutionary history that may not been detected by studies relying exclusively on extant populations. Taking European white oaks (Quercus robur and Q. petraea) as a model, our project aims at tracking past demographic, migratory and selective trajectories in the face of major climatic changes during the Holocene using ancient DNA spanning a full temporal and geographical range. However, since ancient DNA studies on trees are still in their infancy, we first investigated subfossil wood material from different taphonomical and temporal contexts to optimize access to authentic ancient DNA. Optimized aDNA extraction techniques followed by genomic library construction, shotgun sequencing and DNA damage pattern analyses allowed to authenticate 128 ancient oak DNA samples aged between 700 and 9500 years.

Endogenous oak aDNA content varied widely and revealed first ancient oak chloroplast genomes based on which last glacial refuges could be identified.

Molecular adaptation in spruce species

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The rate of adaptive molecular evolution, α, is an important evolutionary parameter and various methods have been developed to estimate it. An important question is whether αis – as one would expect if genetic drift is an important evolutionary force – positively correlated to effective population size, Ne. A related measure to α, the rate of adaptive evolution relative to the rate of neutral evolution, ωa, was estimated in a large number of species and found a positive correlation between estimates of ωa and effective population size (Gossmann et al. 2012). However, in order to control for other forces influencing ωa, estimates for species within the same genus would be preferable. In the present study we will first estimate demographic parameters, including Ne, in four spruce species with varying level of polymorphisms. We will then estimate ωain each of the four spruce species. We will discuss the results in relation to recent findings in other plant and animal species.

Bayesian computation analyses suggest a complex evolutionary history in a fir species complex from central Mexico (Abies, Pinaceae)

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Understanding species' demographic history requires considering both their inherent biological characteristics and the geological and climatic context where they have evolved. Such factors may drive expansion, dispersion and/or fragmentation patterns that may ultimately led to population differentiation, speciation and secondary admixture. Nevertheless, identifying individual processes that originated such patterns might be difficult in long-lived species, like conifers, given that they often overlap and operate at different spatial and temporal scales. This problem may be partially overcome by using genetic variation data at multiple loci in a Bayesian context, which may help identify some of those processes with relative confidence. We addressed this issue in a subtropical fir species complex, Abies flinckii and A. religiosa, distributed in isolated montane populations along an east-west gradient in central Mexico. We examined their divergence and past range dynamics throughout complementary analyses on their nuclear and cytoplasmic genetic variation. Markers surveyed included four nuclear and chloroplast SSRs, together with eleven nuclear and one mitochondrial gene regions; these were genotyped/sequenced in approximately 400 individuals from 20 populations. Individuals clustered into four genetic groups that were consistent with recent taxonomic fir classifications, although significant admixture was inferred in the Colima Volcanic Complex (western Mexico). Taking into account the geological and climate evidence available for central Mexico, we further tested, through approximated Bayesian computations, various divergence and demographic scenarios for these taxa by using the observed genetic structure as a baseline. The most supported scenarios involved an early divergence of two ancestral lineages that further fragmented into various populations with varying levels of more recent gene flow during the last five million years. Our findings highlight the importance of geological and climatic events during the Pliocene and the Pleistocene in central Mexico as putative drivers of species diversification and range shifts, and suggest that selective events might also have being involved in shaping the current Mexican conifer diversity.

Demography at different scales in the wide-spread tropical species Symphonia globulifera (Clusiaceae)

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Spatial genetic structure (SGS) is the result of multiple evolutionary forces (drift, mutation, gene flow, selection) acting over a species range. Depending on the spatial scale, those processes will have different influences on geographic patterns. Thus, different scales can provide complementary information about ecological and evolutionary processes operating on populations and species. Our study is focused on Symphonia globulifera L., a very ancient species which occurs in most of tropical Africa and the Neotropics and that probably dispersed from Africa to America. We used six nuclear microsatellites and chloroplast sequences to assess fine-scale SGS in seven populations from both continents, to contrast isolation-by-distance expectations across populations under distinct biotic and abiotic conditions (topographic features, disperser communities, putative historical events). Whereas in American populations drift-dispersal forces seemed to determine the fine-scale SGS, the African populations showed genetic discontinuities within populations, which was tentatively attributed to more restricted dispersal due to topographical complexity or less efficient disperser communities, but also to secondary contact of isolated gene pools. We also evaluated the wide-range genetic structure and colonization history between continents of S. globulifera, using 4921 single nucleotide polymorphisms (SNPs) obtained by genotyping-by-sequencing from 9 populations in both continents. The moderate number of SNPs obtained despite using a next generation sequencing technique was due to the strong genetic differentiation among populations and between continents, which also caused a probable bias towards low intra-population variability. We employed population genetic (PCA, Bayesian clustering and identification of candidate loci under selection) and phylogenetic analyses to infer spatial genetic structure and detect signals of selection. Intra-continent differentiation was almost as strong as between continents. Populations from Benin and São Tomé (Africa) were genetically intermediate between Central African and American populations, providing information on the biogeographic history of the species. In two French Guiana populations that harboured two distinct morphotypes, distinct gene pools were detected and were broadly congruent with morphotype identity. The genetic differentiation was strongly correlated with geographic distance among populations and suggests that Ituberá (Brazil) and the alternative morphotype in French Guiana could be the most recent lineages in America. We also detected 277 FST outlier loci that could have been affected by selection, over which we are testing the influence of different environmental variables. Our study sheds light onto how a widespread species reached its current distribution and provides insight into the main micro- and macroevolutionary processes (including adaptation) affecting its spatial genetic structure.

Linking demographic history and microevolution in an expanding conifer species using genomic and tree ring data

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The level of local adaptation in widely distributed temperate tree species is remarkably high, and so is gene flow across large distances (Savolainen et al. 2007; Kremer et al. 2012). These observations prompt questions about the mechanisms that allow tree species to quickly respond to selection pressures when establishing into new areas. Previous findings have linked life history traits of trees, such as long juvenile phase and longevity, to a reduced bottleneck effect along colonization routes

(Austerlitz et al. 2000). As a result, newly-founded tree populations may acquire high levels of genetic diversity from the start, which enables them to adapt quickly to new environmental conditions. It is also hypothesized that infrequent long-distance (or frequent medium-range) dispersal events allowed temperate tree species to quickly track the receding Pleistocene ice sheet into newly available habitat (Le Corre et al. 1997). Simulation-based support to these hypotheses is strong, but empirical evidence is scarce.Our research uses Picea sitchensis (Sitka spruce) to empirically test these hypotheses. Picea sitchesis shows strong genetic clines of local adaptation across 22 degrees of latitude along the North-American Pacific coast, and its Northern range is still expanding into South-central Alaska (Mimura & Aitken 2007).One way to characterize the speed of postglacial colonization and bottleneck severity is to use the Approximate Bayesian Computation framework. The expansion models we developed integrate influential tree life-history traits and were tested using an array of genomic markers developed from sequence capture. During this proposed talk I will present our latest results which used key model parameters to date successive population establishment events and to quantify bottleneck severity in the northern Sitka spruce range.I will then present the link between these findings and a different approach to the same question, in which we focused on the front of colonization on Kodiak Island, Alaska. Picea sitchensis reached Kodiak Island about 500 years ago. By sampling trees from 2 to 500 years old and associating tree ages and ring patterns with genotypes (developed from Genotype-by-Sequencing), we can directly monitor the evolution of genetic diversity and structure of the newly established forest. This direct approach complements larger scale statistical inference about population evolution during range expansion.

SESSION 5

KEY NOTE: Christian Maltecca

Causes and consequences of inbreeding: a livestock genomic perspective

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The role of genomic information to constrain inbreeding and monitoring losses of genetic variation has been widely demonstrated. With the correct use of genomic, genetic diversity and inbreeding accumulation can be effectively managed. Management of diversity rests on three pillars. Understanding the basis and consequences of genetic diversity. Managing the population by controlling its effective size. Optimize genetic variability deployed through mating plans. Inbreeding management often rely on the implicit assumption that individuals with the same inbreeding share the same genomic load. Marker information allows instead for regions-specific stretches of homozygosity causing inbreeding depression to be identified. Yet, these regions are expected to be at a low frequency so that traditional association methods based on estimating dominance effects and geared toward common variants lack statistical power. Methods that exploit the fact that longs runs of homozygosity (ROH) are enriched with deleterious variants can have greater power in identifying haplotypes linked to inbreeding depression. Here we present results from multiple livestock species as well as simulated data that show the power of alternative genomic similarity metrics to curtail inbreeding accumulation and identify potential deleterious haplotypes.

Use of molecular markers to enhance genetic gains in the maritime pine breeding program

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Maritime pine breeding in France started in the sixteens with the selection of about 600 plus trees in the Landes forest. A classical recurrent selection strategy has been implemented to improve both growth and stem straightness. Polycross trials are used for backward selection in order to establish seed orchards whereas the breeding population is renewed through bi-parental mating. Molecular markers can be of great help to enhance genetic gains in breeding. We explore three main

implementations of molecular markers in the maritime pine breeding program.The first one is DNA fingerprinting to check identities and pedigree in the clonal archives. A SNP set has been developed to reach this goal, and, since 2016, clonal archives identities are progressively controlled. An average error rate of 15% has been highlighted. These pedigree errors can be corrected to increase breeding value accuracy.The second one is the use of molecular markers to recover pedigree when at least one parent is unknown. A pilot study has been carried out in a polycross trial which opens opportunities for new breeding strategies such as "polymix breeding with paternity recovery". This strategy will be implemented in the maritime pine breeding program to quickly add new genetic variability in the breeding population.And, finally, two proof-of-concept studies of genomic selection were established and have shown medium to high accuracy for the two main selection criteria: growth and stem straightness. Advantages and limits of genomic selection will be discussed in the context of the maritime pine breeding program.

Accuracy and precision of genetic parameters using the single-step BLUP method with varying genotyping effort in open-pollinated Picea glauca

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Maximizing genetic gain at minimal cost is a major goal of tree improvement programs. Observed genetic gain depends primarily on the accuracy and precision of the estimated genetic parameters obtained from progeny trials. Thus, the quality of these parameters are contingent on the accuracy of the individual tree field performance records and their genealogical relationships. The use of genomic marker information can improve estimates of relatedness over the expected values by quantifying an actual proportion of alleles shared by individuals, however, the cost of genotyping every individual is still prohibitive in large forest tree improvement programs. Here, we investigated the use of a blended relationship matrix (H: HBLUP) (Legarra et al. 2009; Misztal et al. 2009; Christensen and Lund 2010) and compared it to traditional ABLUP analysis (Henderson 1984) implemented in ASReml-R (Butler et al. 2007). The blended HBLUP method reflects the realized genomic relationship information (G) of a subset of genotyped individuals to the full traditional average numerator matrix (A) making it possible to combine both genotyped and non-genotyped Individuals in a single analysis. Thus, the requirement for genotyping the full breeding population is unnecessary.We investigated this approach using phenotypic (22-year tree height and X-ray wood density) and genomic markers (6716 SNPs) for 1,694 white spruce (Picea glauca) progeny representing 214 unrelated open-pollinated (OP) families growing in a single progeny test site in Quebec, Canada (Beaulieu et al. 2014a). The effect of various levels of within family genotyping effort (0, 25, 50, 75, 100%), and the use of two different genomic relationship matrices (VanRaden 2008; Dodds et al. 2015) were assessed on the basis model fit, accuracy and precision of genetic parameter estimates, breeding value rank changes, and cross-validation prediction accuracy of breeding values. The results showed that the HBLUP method considerably improved the accuracy and precision of breeding value estimates over traditional ABLUP analysis by accounting for Mendelian sampling variation. Further, the genomic information helped in reducing the heritability bias commonly observed in OP progeny trials as it revealed hidden relatedness, inbreeding, as well as possible pedigree errors. Overall, the effect of increasing within family genotyping effort improved all aspects of HBLUP compared to ABLUP. The data for this study was obtained freely from the Dryad Digital Repository (Beaulieu et al. 2014b).

Using genomic information to increase resilience of forest tree plantings

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The world's forests are being challenged by environmental change due to global warming; increasing exposure to exotic competitors, pests and diseases; and human population pressures. How we take

genomics beyond knowledge discovery to aid the development and implementation of adaptation strategies to such change in the required time frame is a major challenge. We here describe a pathway we are exploring with this objective. While identification of causal genes underlying adaptation and mass screening of germplasm would be ideal, it is not practical. Instead, we are investigating a broad approach that can be applied in many species. We are using population genomics to identify and weight the key environmental drivers that have shaped local adaptation. First, genome-wide scans are used to identify a suite of markers showing signals of disruptive selection. The environmental variables most closely aligned with the variation in this putative adaptive space are then identified. The major environmental correlates are used to develop spatially explicit and biologically-relevant fitness surfaces for contemporary and future climate projections. While functional trait analyses and field trials will provide validation of these models in the long-run, in the absence of better information the models can be used as transfer functions to guide seed source choice or identify components of native gene pools most at risk of future maladaptation. Such an approach is particularly valuable for forest tree plantings, where long generation times and size of forest trees make measurement of life-time fitness difficult.We demonstrate this approach in the context of climate-adjusted provenancing for ecological restoration (Prober et al. 2015), using several eucalypt species in Australia. Traditionally, local provenances have been favoured due to the 'local is best' paradigm. However, this is increasingly being challenged due to issues of seed supply and quality (e.g. inbreeding), site modification and climate change. Climate-adjusted provenancing (analogous to assisted migration) advocates supplementation of local provenance with germplasm from along a gradient of change (e.g., increasing aridity), with a bias towards provenances that exist in projected analogous climates. The premise of this strategy is to capitalise on inherent genetic diversity and adaptive capacity across a species' range. Apart from identifying and weighting putative climate drivers of adaptation in the target species, our genomic approach has potential to flag whether climate-adjusted provenancing may be compromised by other environmental drivers of adaptation, such as soils.

Genomic Selection in Douglas-fir (Pseudotsuga menziesii)

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Genomic selection (GS) can offer unprecedented gains to forest tree breeding, especially for late expressing traits and those with low heritability. Here, we used: 1) exome capture as a genotyping platform for 1,348 Douglas-fir trees representing 42 full-sib families growing on 3 sites in British Columbia, Canada and 2) growth and wood quality traits as phenotypes. Two GS predictive methods (Ridge Regression Best Linear Unbiased Predictor (RR-BLUP), and Generalized Ridge Regression (GRR)) were used to assess their predictive accuracies over space (within site, cross sites, and multi-site to single site) and time (age-age). Additionally, an independent validation population consisting of 490 individuals were used to verify the original 1,348-tree models. The RR-BLUP and GRR models produced similar predictive accuracies across all traits. Within-site GS prediction accuracies were high and generally better than the combined sites and multi-site to single-site predictive accuracy fell in between these two values. Cross-site predictions had the lowest predictive accuracy, reflecting genotype x environment interactions. The independent validation produced poor predictive accuracies, most likely due to the breakdown of linkage disequilibrium over successive generations, leading to the inability of the derived models to produce reliable predictions and ultimately confirming the importance of regular GS models update in response to the changing genetic architecture of the population as a whole over multiple generations.

A tale of two pathogens: Accelerating restoration of the American chestnut using genomic selection

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The American chestnut (Castanea dentata) is a unique example of a tree species, decimated by exotic pathogens, that is on the verge of restoration through breeding and biotechnology. The root pathogen Phytophthora cinnamomi, which was introduced to the southeastern U.S. in the 1700s, eradicated American chestnut from low elevation forests in this region in the 1800s. The chestnut blight fungus Cryphonectria parasitica, which was introduced from Asia in the mid-1800s, killed nearly 3.5 billion chestnut stems throughout the eastern U.S. by the 1950s. Over the last thirty years, The American Chestnut Foundation (TACF) has used backcross breeding to introgress alleles of major effect for disease resistance from Chinese chestnut (Castanea mollissima) into American chestnut. Third backcross hybrids (B3s), which inherit an average of 15/16ths of their genome from American chestnut, were intercrossed to generate a genetically diverse, segregating population of B3-F2 trees that are expected to contain a subset of individuals homozygous for alleles with major effect on disease resistance. Currently, TACF has advanced two sources of resistance, derived from the 'Clapper' and 'Graves' first backcross trees, to B_3-F_2. Over 60,000 B3-F2s from these sources have been planted in seed orchards at TACF's Research Farms in Meadowview, VA since 2002. After artificially inoculating seedlings with C. parasitica and culling individuals with significant canker expansion, 5,000 to 9,000 trees will remain from which to make the final selections of ~500 trees most resistant to C. parasitica or P. cinnamomi. Since 2009, ~400 B3-F2 mother trees have been progeny tested for C. parasitica resistance and ~200 have been progeny tested for resistance to P. cinnamomi. To increase the speed and accuracy of selection in seed orchards, genomic prediction models for resistance to both pathogens are being developed. Using restriction site associated DNA sequencing, ~31,000 SNPs were genotyped in 600 Graves and 400 Clapper B3-F2s. The SNP genotypes were used to infer paternity and realized genomic relationships among the B3-F2s. Results will be presented on the accuracy of genomic prediction models as compared to prediction from pedigree relationships alone. Furthermore, comparative association mapping of blight resistance in 'Clapper' and 'Graves' will provide insight into whether these sources contributed unique alleles for disease resistance to their progeny. Genomic selection holds promise to greatly accelerate selection for disease resistance in American chestnut backcross hybrids.

KEY NOTE: Aaron B.A. Shafer

Conservation [and] genomics of free-ranging populations

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Governments acknowledge the importance in stemming the loss of biodiversity, and conserving genetic diversity is a strategic goal of the UN's Convention on Biological Diversity. Genomic approaches have been touted as a promising tool to support such aims, with scaling up to genome-wide data thought to improve upon the traditional conservation genetic inferences and provide qualitatively novel insights for management decisions. However, the generation of genomic data, subsequent analyses and interpretations remain challenging, nuanced, and often far-removed from on-the-ground conservation issues. Here I highlight some of the major hurdles limiting the application of genomic data to conservation biology and offer solutions and outlook for the future.

Assessing vulnerability to support conservation management. An example with two Mediterranean pines

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The negative impact of climate change on forest systems is leading to an increased awareness of the importance to implement conservation plans in national and international policies. The selection of specific conservation strategies (ex situvs in situ conservation) requires a sound knowledge on theextent to which species are threatened due to vulnerability to climate change. Thus, we propose a novel, systematic and easily implementable methodology to assist the definition of conservation strategies for broadly distributed species by assessing two major components of vulnerability: exposure to climate change (approached by risk of habitat loss and accounting for future climate uncertainty) and adaptive capacity (approached by genetic diversity). Our method is illustrated by two iconic Mediterranean conifers, Aleppo and maritime pines, alleviating the underrepresentation of Mediterranean ecosystems in precedent conservation studies. Further, we assess the adequacy of the current European conservation network based on Dynamic Conservation Units (DCUs) for these two species.We consider previously genetically defined clades as main conservation units. We fit species distribution models for each clade individually and project them into current climate and 42 different future climate predictions representative of 2050. We create future suitability maps to assess risk of habitat loss and possible translocation areas depending on the number of future climate predictions that project the clade to be suitable in the future. Based on the habitat loss evaluation, we propose the most adequate conservation strategy: in situ, in situ with monitoring and ex situ conservation. In the last case, we distinguish between translocation and conservation in germplasm banks. We detect a very different exposure among clades distinguishing southern-most clades for maritime pine and Greek and Moroccan clades for Aleppo pine as those most highly exposed.Finally, we identify objectives to increase the efficiency of the current conservation network: (i) the selection of dynamic conservation units (DCUs) should adequately represent in a collective manner all existing clades (at the moment two and three clades respectively for Aleppo and maritime pine remain unrepresented and the representation of the rest is largely unbalanced); (ii) achieving a low risk of habitat loss in the future should be considered as an additional minimum requirement for a population to become a DCU (currently only 28% of the analysed DCUs fulfils this criterion).

Collecting seeds for ex situ conservation of forest trees: how much and from where ?

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Changing environments and global climate threaten many forest trees. In response, conservation, restoration, and breeding organizations are initiating or expanding ex situ seed collections from forests (e.g. the United States Bureau of Land Management, United States Forest Service, and the Millennium Seed Bank at Kew Gardens). While it is advisable to capture as much phenotypic and genetic diversity as possible, quantitative advice about how much and where to collect is lacking. This knowledge is urgently needed especially for rapidly declining species. I demonstrate a new approach to optimize sampling protocols for collecting seed (Hoban and Strand 2015, Hoban and Schlarbaum 2014). I use spatial, demographic and genetic data from three species, and simulated data under an individual-based model, to design collections that maximize genetic diversity while minimizing collection size. I find that (as expected based on theory) reproduction and dispersal traits significantly influence the genetic diversity captured in seed collections, as does perennial vs. annual life history. For example, a highly self-pollinating, low dispersal species needs sample sizes five times larger than current guidelines. I also discuss the extent to which existing collections have optimally collected in the past. I then briefly demonstrate how other aspects can influence seed collections design, like recent population history. Results show that collectors can and should customize their sampling protocols for

the target species, rather than use commonly implemented “rules of thumb.” This approach will help to efficiently and effectively achieve breeding, restoration and conservation success.

Phylogenomics of the ash genus to discover genes conferring low susceptibility to ash dieback and emerald ash borer

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Ash populations in Europe are currently devastated by ash dieback (Hymenoscyphus fraxineus) and the emerald ash borer (Agrilus planipennis) is destroying North American ash populations. Having recently sequenced the genome of Fraxinus excelsior (European Ash) we are now sequencing the genomes of 35 other species of the genus Fraxinus. Several of these species, particularly those from east Asia, are reported to have low susceptibility or resistance to ash dieback and the emerald ash borer. We are testing these species for susceptibility to ash dieback and emerald ash borer. It appears that low susceptibility to the pathogen and the pest occur convergently in different clades of the genus Fraxinus. We are therefore seeking to find genes in evolved in low susceptibility by building genus-level gene trees for thousands of genes, seeking those with topologies that are incongruent with the consensus species tree but fit with the distribution of low susceptibility in the genus. We hope that this will provide a rapid method for identifying candidate genes that may be transferred between species by hybridisation or cis-genetics.

Worldwide translocation of teak – origin of landraces and present genetic base

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Teak (Tectona grandis Linn. f.) with its natural distribution area across parts of India, Myanmar, Thailand and Laos (Kaosa-ard 1981) is worldwide known for excellent timber. Consequently teak is one of the most expensive hardwoods grown in plantations (FAO 2015). Furthermore, with a global estimate of approximately 4.35 million ha plantations (Kollert and Cherubini 2012) including plantings in 65 countries outside of its native range (Koskela et al. 2014), teak is today one of the most important plantation species in the World. The process of transferring teak germplasm around the globe started more than 100 years ago, but the genetic origin and diversity behind these introductions and potential effects of local landrace formation have so-far been poorly investigated. However, a recent study of genetic variation within and among populations from the total natural distribution area of the species revealed strong genetic structure and large differences in genetic diversity among regions (Hansen et al 2015). In the present study we use this ‘global genetic reference map’ to infer on the likely genetic origin of major landraces with special emphasize on tropical America and we compare the genetic diversity among landraces to the diversity levels present in natural populations from their likely origin. We find that of 17 investigated land races from Indonesia, Africa and tropical America the majority most likely origins in Southern Myanmar or North-East India; the genetic analysis only suggests some of the Indonesian landraces to have likely origin further east in Thailand. None of the studied land races seemed to have origin in South and West India or in Northern Myanmar. The 7 landraces from tropical America were genetically quite similar and may therefore originate from a single introduction of teak into Central America in the beginning of the 20th century as suggested by historical records. Differences in genetic diversity among landraces were observed – but only one landrace from Africa showed extremely low genetic diversity indicating a major bottleneck. We

conclude that variation in diversity levels among teak landraces probably reflect their areas of genetic origin rather than sever founder effects created during the introductions out of Asia to Africa and Americas.

Assisted diversification for the Anthropocene

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Many of Earth's species are threatened with extinction, primarily due to habitat loss. The most daunting threat is actual habitat extinction as fundamental environmental and ecosystem processes are altered and shifted by humanity's activities. During previous global extinction events, plants have suffered less than animals and their flexible reproductive behavior and participation in syngameons may hold a key to this macro-resilience. We should exploit inter-fertility among lineages to enhance their phenotypic diversification. Given accelerated evolution of environmental conditions and habitats, we should similarly accelerate plants' natural coping behaviors. A experimental program to create diversity groves by enhancing genetic experimentation within plant lineages and maintaining variants should facilitate adaptation to the unprecedented habitats of the imminent Anthropocene. In this talk, I will describe an experimental approach to generate a wide range of genotypes and phenotypes for study in a diversity grove, starting with oaks as a case study. Using our growing knowledge of evolutionary and physiological dynamics, this diversity grove could provide a mechanism for rapid adaptive introgression and breaking down covariance in traits to produce novel phenotypes for novel environmental futures. This experiment would serve as a 'fail-safe' standing stock of diversity against unforeseeable challenges in the Anthropocene.

SESSION 6

KEY NOTE: Stephen P. DiFazio

Genome Dynamics and Sex Determination in the Salicaceae

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The Salicaceae family, including Populus and Salix, is a powerful model system for studying the influence of genome structure on the evolution of adaptive traits. A comparison of synonymous nucleotide substition rates between Populus and Salix demonstrates that the Salix lineage has accumulated more polymorphisms. This elevated evolutionary rate is recapitulated in higher rates of genome fractionation in Salix following the shared Salicoid genome duplication. The two genera have numerous contrasting phenotypic characterisitics, including growth form, pollination mode, and generation time. Furthermore, although both species are primarily dioecious, they appear to have different sex determination loci. Sex determination in Populus is controlled primarily by loci on chromosome 19 across all species that have been studied thus far. In contrast, it is now clear that sex determination occurs on Chromosome 15 in Salix purpurea, based on mapping in an F2 intraspecific cross as well as in an association population of unrelated individuals,. This is quite surprising, especially given the high collinearity of the vast majority of the Populus and Salix genomes. Furthermore, examination of the genotypes of sex determination loci in male and female trees suggests that Populus trichocarpa has a predominantly XY sex determination system, while Salix purpurea has a ZW system. A comparative analysis of the sex determination regions of the two genera reveals many shared characteristics, including structural complexity, high repeat density, and suppressed recombination. Ongoing questions include whether there are shared mechanisms of sex determination in the two species, the extent of sex dimorphism, the role of pollinator attraction and defense in sex chromosome evolution, and the presence of sexually antagonistic genes in the sex determination regions.

The Chinese chestnut (*Castanea mollissima*) genome

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The accidental introductions of the chestnut blight fungus (Cryphonectria parasitica) to North America in 1904 and to Europe in 1938 led to devastating epidemics devastating American chestnut (Castanea dentata) and European chestnut (C. sativa) populations. Asian chestnut species, which evolved resistance to the sympatric chestnut blight fungus, are of interest as donor species for transfer of resistance genes to C. dentata and C. sativa through breeding or transformation. In the US, crosses to Chinese chestnut (Castanea mollissima) formed the foundation for the development of blight resistance in American chestnut. To better understand the genetic basis of blight resistance and to provide tools for advancing chestnut breeding, we have sequenced the genome of Chinese chestnut, with particular focus on the three major blight resistance QTL. A first draft genome assembly (v.1.1) of 41,270 scaffolds covering 724.4 Mb of the estimated 800 Mb chestnut genome was obtained for the cultivar 'Vanuxem'. A consensus of 38,146 genes was predicted and annotated, and confirmed with expression data. BAC clones tiling the three major blight resistance QTL were sequenced to great depth. Across all three QTL regions, 782 genes were annotated with a diversity of molecular functions and biological processes, including approximately 200 genes in the GO stress-response classification, and 15 "defense response" genes. The version 1.1 genome scaffolds, QTL scaffolds, predicted genes, transcripts and proteins were released to the public in January 2014, at the website http://hardwoodgenomics.org/chinese-chestnut-genome. More recently, a new assembly (version 2.2) of the Chinese chestnut genome was obtained covering 98% (784 Mb) of the genome in 14,358 scaffolds, of which 5,745 were ordered along the chestnut genetic linkage map into 12 "pseudochromosomes" covering 725.3 Mb. Validations of the pseudochromosomes are underway using additional genetic maps, PacBio reads, and cytological assignments of tagged BAC clones by FISH. Whole genome sequence from C. alnifolia, C. crenata, C. henryii, C. ozarkensis, C. sativa, C. seguinii, and a selection of C. dentata, C. mollissima, and backcross genotypes have been mapped to the reference pseudochromosomes to assess synteny and introgression. We hope that the Chinese chestnut genome will provide a solid reference for studying genome evolution and for advancing disease-resistance breeding. The Chinese chestnut genome project was supported by The Forest Health Initiative (http://www.foresthealthinitiative.org).

Preservation of genome structure and gene sequence across the Fagales utilizing peach as an outgroup

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New genomic resources have been rapidly emerging for tree species in the Fagales, including draft reference genome sequences, dense genetic maps and transcriptome sequences. However, no Fagales species yet has a publicly available, finished genome with the majority of gene space anchored in pseudochromosomes. This prevents progress in identification of candidate genes in QTL regions or near phenotype-associated genetic markers and in cross-species comparisons of orthologs and gene families. We have developed a translational genomics technique to alleviate this problem. By leveraging the extensive macrosynteny between Chinese chestnut and peach, we have created a peach-guided chestnut genome assembly where chestnut contigs are placed in pseudochromosomes, i.e. "super-scaffolding" of chestnut genome segments against the peach reference genome. Evidence

leveraged for scaffold placement and synteny includes the sequencing similarity from the finished peach genome to chestnut genetic map markers, the chestnut physical map BAC-end sequences, and chestnut genes derived from the draft chestnut genome assembly. This construction has already proved fruitful in the search for candidate genes in chestnut blight resistance QTL regions (Staton et al., 2015). The analysis has been expanded to compare the draft genome scaffolds from additional important Fagales lineages, the pedunculate oak (Quercus robur) (Plomion et al., 2016) and the English walnut (Juglans regia) (http://dendrome.ucdavis.edu/ftp/Genome_Data/genome/Reju/). The results reveal that the conservation of genomic structure and sequence found in chestnut when compared to peach is also present in other members of the Fagales family.

Oak genome sequencing and evolution

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Oaks are among the most impressive trees. Their long-lived nature, symbol of strength and endurance; their biological dominance, characterized by a species-rich assemblage of organisms below and above ground, make them keystone species of major ecosystem functioning. These species are ecologically and genetically very diverse, growing in extremely variable conditions throughout the northern hemisphere where they provide a wide range of environmental and economic services.To obtain a WGS assembly of Quercus robur (~ 750 Mb/C), we used a combination of i/ short reads (Illumina) from size-selected sequencing paired-end and mate-pair libraries, ii/ reads of medium length (454-Roche), iii/ synthetic long-reads (Illumina) and iv/ BAC ends (Sanger). Synthetic long-reads made it possible to better separate both haplotypes of the selected tree genotype, owing to the accumulation of polymorphisms in this highly heterozygote species. Sequences were assembled into 8,827 scaffolds (>2kb) representing 1.45 Gb, with an N50 scaffold length of 0.82 Gb. Merging both haplotypes further helped to improve contiguity. The haploid genome assembly (1,409 scaffolds), consisted of ~ 810 Mb with an N50 scaffold of 1.34 Mbp, 88% of which (represented by 876 scaffolds) were anchored to the 12 chromosomes using a high density SNP-based linkage map and a syntenome strategy using Prunus persica as a pivotal genome sequence.We then investigated the evolutionary history of the modern oak genome in comparing the 26,768 annotated genes covering 714.4 Mb anchored on the 12 pseudomolecules to that of available eudicot genomes. From the eudicot ancestor structured in 21 chromosomes and containing 7,072 protogenes, we found that the oak genome did not experience lineage-specific whole genome duplication, and harbors relics of the known ancestral (gamma) triplication (hexaploidization) shared by the eudicots. From this ancestor, the modern oak genome has been shaped trough intense chromosomal rearrangements involving ancestral chromosome fusions and fissions. At the gene level, 92 families have expanded specifically in oak, compared to fifteen other eudicot species, consisting in major gene functions operating as a vehicle for oak adaptation.

Annotation of the oak genome sequence and associated bioinformatic resources

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The large, complex and highly heterozygous genome of pedunculate oak (Quercus robur) was sequenced using a whole-genome shotgun approach [1] . Roche 454 GS-FLX sequence reads were assembled into contigs and combined with Illumina reads from paired-end, mate-pair libraries and true synthetic long reads to build a total of 8,827 scaffolds (1.46 Gb total size; N50=821 kb). Both haplotypes were merged into an haploid version and 12 pseudomolecules were established using a high-density linkage map [2] combined with a syntenome approach using the peach genome sequence.The structural (Transposable Elements (TEs), genes, ncRNA) and functional annotation of automatically predicted genes relies on powerful and robust pipelines: (i) REPET package [3] [4] was first used to de novo detect, classify and annotate TEs representing about 50% of the genome; (ii) Eugene was trained and launched to integrate ab initio and similarity gene finding software to finally predict 43,240 genes including 29,665 highly confident gene models; (iii) ncRNA were predicted using feelcn (lncRNA), similarities against databases and small RNAseq data analysis (miRNA), RNAmmer (rRNA), tRNAscan-SE (tRNA) and Infernal package (other non-coding RNA) (iv) A functional annotation pipeline mainly based on Interproscan to search for patterns/motifs and Blast based comparative genomics was launched onto the 43,240 predicted proteins. The assignation of a provisional definition for predicted protein according to the results of the most reliable tools and their occurrence in Oak annotation was produced (D. Goodstein method, personal communication). We will present here these pipelines and the results of this annotation.We also set up an integrated genome annotation system (dedicated to oak) based on GMOD web interfaces such as WebApollo/JBrowse and Intermine to make these data available under a user-friendly environment. This system allowed experts to analyze their respective protein families of interest and curate/validate gene structure. We will also present the interoperability between these genomic data and genetic data produced in Quercus (SNPs, linkage maps, QTLs) available in GnpIS [5] an information System for plants.All together these resources provide a framework to study the two key evolutionary processes that explain the remarkable diversity found within the Quercus genus: local adaptation and speciation.

KEY NOTE: John MacKay

Gene Copy Number Variations in spruce (Picea spp.): detection and potential roles in evolution

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Gene copy number variations (CNVs) have been linked to many heritable conditions in human and to evolution in model and crop plants but little is known of their frequency and potential contributions to evolution in forest trees. This knowledge gap is common to most wild species as established CNV detection approaches usually use high quality reference genome assemblies. Here, we developed two robust approaches to overcome this technical barrier and investigate CNVs in spruce trees. We developed comparative genomic hybridization on arrays (aCGH) and implemented an approach to reanalyse data obtained from Illumina SNP genotyping arrays. Each of the methods targeted independent sets of 14,000 genes and were used to analyze full-sib families, with controls for false discovery. We detected both common or inherited CNVs and rare CNVs classified as de novo mutations.

Whole genome hybridizations (aCGH) were obtained for 80 individuals of white, black and interior spruce, the latter representing a natural hybrid, and identified hundreds of CNVs in each pedigree. The entire set represented 3,612 distinct CNV genes which were enriched in stress and defense responses functions. The genes were distributed throughout the genome based on their position on a spruce genetic map, suggesting numerous and widespread structural variation events. The hybrid spruce had much fewer CNVs which could mean that the mixture of different genomes within a single species decreases CNVs, potentially reducing the adaptive variability and evolvability of hybrids. In an independent study, we reanalyzed genotyping array data in 55 full-sib families and over 3500 individuals. Nearly 150 CNVs meeting high stringency and repeatability criteria were studied in detail. We found that this approach was particularly effective at detecting gene copy number losses. Many de novo CNVs were detected and these allowed us to estimate mutation rates, which we found to be highly variable between genes and correlated with expression. We also observed that around half of the inherited CNVs were associated with patterns of transmission distortion.
Taken together, our results indicate that CNVs are over represented in gene families of particular relevance for adaptation and are associated with or influenced by evolutionary processes including hybridization, selection and high mutation rates. Future research is needed to directly test whether CNVs are linked to phenotypic variation which may shed new light into the molecular basis of quantitative traits.

A systems biology approach for identifying candidate genes involved in the natural variability of biomass yield and chemical properties in black poplar

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Lignocellulosic biomass is a renewable resource of interest for biorefinery. However, current poplar varieties have not been selected for this specific purpose. The factors affecting biomass yield and chemical properties need thus to be studied. With this objective, we have initiated a systems biology approach, integrating genomic, transcriptomic and phenotypic data in natural populations of black poplar (Populus nigra). Up to now, we have focused on a subset of 12 genotypes from 6 populations and trialled in a randomized complete block design located at INRA Orléans, France. The transcriptome of 2 biological replicates of each genotype has been explored through RNA sequencing (RNAseq) of pools of young differentiating xylem and cambium. Additionally, biomass yield was evaluated through measurements of height and diameter on 6 replicates of each genotype across several years and rotations, while biomass propertieswere assessed through chemical analyses of lignin, cellulose and hemicellulose concentrations as well as saccharification potential on 3 replicates of each genotype. The resulting data were used to build a weighted gene co-expression network and identify gene modules whose expression was correlated with biomass yield and/or quality at the genotypic level. Remarkably, the largest module (1,460 transcripts) was significantly associated with klason lignin content and displayed an enrichment in genes involved in secondary cell wall formation. Four candidate genes from this module were further selected to validate the detected quantitative trait transcripts (QTTs) on 2 new replicates of the 12 genotypes using RT-qPCR. The resulting expression levels were significantly correlated to those previously quantified by RNAseq and to the klason lignin content in the wood samples. These results demonstrate the interest of our approach, and thus open some prospects towards the identification of new candidate genes whose functions remain to be elucidated.

Pinus sibirica and Larix sibirica whole genome de novo sequencing

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The Siberian larch (Larix sibirica Ledeb.) and Siberian pine (Pinus sibirica Du Tour.) nuclear and organelle genomes are being de novo sequenced in the Laboratory of Forest Genomics at the Genome Research and Education Center of the Siberian Federal University using Illumina HiSeq 2000 and MiSeq, and their first draft genome assemblies were generated (http://genome.sfu-kras.ru/en/main). Estimated genome size was 12.03 Gbp for Siberian larch and 28.90 Gbp for Siberian pine. DNAs isolated from needles, single megagametophytes and a haploid tissue culture of a reference larch tree and from needles and single megagametophytes of a reference pine tree were used to generate multiple PE libraries with 250, 400 and 500 bp long inserts and MPE libraries representing 3 and 5 Kbp long fragments. We tested CLC Assembly Cell, ABySS and MaSuRCA assemblers that were used in the similar conifer genome sequencing projects. The assembling was done using the IBM x3950 x6 server with 96 cores and 3 TB RAM. ABySS was the most stable, but the best assemblies were generated by CLC Assembly Cell. The best Siberian larch genome assembly was ~5.5 Gbp long (that is 46% of the expected complete genome length) with N50 for contigs equaled 1947 bp. Almost all Siberian pine short reads were successfully mapped to the draft genome assembly v1.0 of closely related sugar pine (Pinus lambertiana Dougl.) generated in the PineRefSeq project (http://pinegenome.org/pinerefseq) covering more than 80% of the assembly (~21.26 Gbp). Thus, the reference-based together with de novo assembly approaches resulted in a draft genome assembly of Siberian pine with a total length of ~22.9 Gbp (79% of the expected complete genome length) with N50 for contigs equaled 2352 bp. About 80% of Siberian larch and pine nuclear genomes consisted of highly repetitive DNA. For the first time the chloroplast genome of Siberian larch has been assembled and annotated. For Siberian pine we completed the partial chloroplast genome assembly available in Genbank (FJ899558.1) by closing all gaps. The draft assemblies of mitochondrial genomes for these species have been also generated. The larch transcriptome assembly consisted of 43717 unigenes with a total length of ~26 Mbp. The longest unigene was 8512 bp; N50 = 1330 bp, and the number of unigenes longer than 1 Kbp was 6919. The obtained transcriptome assembly was similar to other published conifer transcriptomes. This study was supported by Research Grant No. 14.Y26.31.0004 from the Government of the Russian Federation.

A new Genomic resource for Populus nigra and its deployment for genetic studies

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Black poplar (Populus nigra L., Salicaceae) is an Eurasian native species distributed within fluvial corridors. As a pioneer species, P. nigra plays an important role in the establishment of riparian ecosystems. The species has important adaptive performances that have promoted Black poplar as a parental pool in interspecific breeding programs world-wide. Until recently, there is no genomic resource in black poplar to support genetic diversities studies and breeding. INRA, University of

Southampton, University of Udine and IGA were collaborating to develop genomic resources and genetic tools for black poplar. SNPs were discovered using whole genome sequencing of 51 individuals relevant of the genetic diversity of an association population covering the range of the species in Western Europe. Four individuals were selected and sequenced at coverage > 25X and 47 at coverage <25X. We had two main objectives: to maximize the genetic variation among individuals and to identify informative SNPs. More than 189 000 SNPs were identified within 15 known QTL regions related to rust resistance, wood properties, water use efficiency and phenology, 2 916 expressional candidate genes for the same traits, and 1 732 genes spread out on the genome. Because many more SNPs were detected than needed, a set of stringent parameters was applied to filter 10 331 loci for the construction of a 12K Infinium BeadChip array (Faivre Rampant et al, 2016). This new array was employed to genotype more than 1 000 unrelated individuals and progenies. The high SNP call rate over 90% provided valuable information on population genetic structure. The structure pattern was consistent with the geographical distribution of the populations under study. However, admixture is an important feature in French populations. Moreover a high rate of clonality was found in populations from Netherlands and Germany. Most polymorphic SNPs had a Minor Allele Frequency greater than 0.05, showing that the array is suitable for association studies. The array data were also used to estimate linkage disequilibrium. The r2 fell to approximately one half of the initial value within 5 to 7 kb. This new information is important to develop further whole genome association in P. nigra. The 12K Infinium BeadChip array is the first genotyping resource for black poplar, and examples of current applications in diversity studies, pedigree validation, genetic mapping, genomic evaluation and natural-population-based genetic association will be presented. It is also considered as gold standard to fine-tune parameters for SNP calling from RNAseq and whole-genome sequencing data.

Genomic marker development for the study of the drivers of species diversification in neotropical Palms (Geonoma)

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Understanding the drivers of species diversification is of fundamental interest in evolutionary biology and is key for understanding the mechanisms underlying the origin and maintenance of biodiversity. The genus Geonoma is one of the most diverse palm genera in the neotropics with 68 species distributed from sea level to >3'000m in Central and South American rain forests (Henderson 2011). High intraspecific variation is common in this genus, and 18 species complexes are divided into 90 subspecies. For these reasons, the genus Geonoma offers a powerful model for testing the relative roles of geography, dispersal limitation, ecological and intrinsic factors in driving divergence of palm populations and species. With the rapid advances of next generation sequencing technologies, genome-wide patterns of recent speciation have been successfully characterized in emblematic systems such as cichlid fish, sticklebacks, Heliconius butterflies, or sunflowers. In Geonoma palms, genomic studies at the species and population levels are hampered by a lack of genomic markers suitable for the genotyping of large numbers of Geonoma taxa that diverged up to 18.5 My (Roncal et al. 2010). To fill this gap, we used a whole genome sequencing approach to develop target sequencing for 4'247 genome regions of 1'300bp length in average, including 4'114 genes and 133 non-genic regions. These markers were chosen to cover a wide range of mutation rates allowing future studies at the genus, species and population levels, i.e. across micro- and macro-evolutionary time scales. Special emphasis was given to the avoidance of large indels and duplications during marker selection. A total of 1'000 palm samples covering more than 90% of Geonoma species and 15 Central and South American countries will be genotyped using this methodology. Phylogenomic, phylogeographic and population genomic approaches will then be used to study the drivers of species diversification in this clade.

Forest tree GnpIS: an information system dedicated to forest tree genetics, genomics and phenomics

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Due to the major technological advances both in genomics and phenomics, it is now possible to quickly obtain large amounts of data at low cost. One of the consequences is the critical need for data management, with the opportunity to make these datasets interoperable, thus enhancing their reuse and enrichment. GnpIS[1] is an original information system (IS) able to manage these data. It was designed to integrate and link genetic, genomic, phenomic and environmental data into a single environment, allowing plant (crops and forest trees) and fungi researchers to store, query and explore information from different angles. Here I will present the "Forest tree GnpIS", a GnpIS focused on forest tree data.The forest tree resources are accessible through a web portal (https://urgi.versailles.inra.fr/gnpis/). This main entry point is a google-like search, a tool using keywords for data discovery. The bird's eye view obtained allows navigation through the data with dedicated tools facilitating more specific queries and data retrieval. Cards were developed to gather all representative information on major elements (accession, markers, trials …). This IS is regularly improved with new functionalities answering specific needs raised by scientists and released several times a year. Data are supplied by local sources (files and databases) produced and managed by research teams. In order to make data submission easier, workflows are implemented to automate data flow: 1) extraction from local sources and insertion in GnpIS, 2) extraction from GnpIS and insertion into international IS (such as Evoltree eLab).Ash, pine, spruce, willow, poplar and oak data (genetic resources, phenotypes, genotypes, polymorphisms and genetic maps) have been integrated into this forest IS (https://urgi.versailles.inra.fr/Species/Forest-trees/Database-overview). It is possible from a genome browser and its annotation to access genetic maps results (via markers and QTL) and from a QTL for a trait of interest to get the phenotyping results of this trait and to select the accessions with the most interesting results. To refine your selection, you can study the accessions pedigree and genotyping results. Integration of the data produced within the French Common Garden network (over 1,000 trials with genotypes gathered from ~15 species) is in progress and data from other species are expected soon.

Implementation of the realized genomic relationship matrix to open-pollinated white spruce family testing for disentangling additive from non-additive genetic effects

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The open-pollinated (OP) family testing combines the simplest known progeny evaluation and quantitative genetics analyses as candidates' offspring are assumed to represent independent half-sib families. The accuracy of genetic parameter estimates is often questioned as the assumption of "half-sibling" in OP families may often be violated. We compared the pedigree- versus marker-based genetic models by analyzing 22-year height and 30-year wood density for 214 white spruce (Picea

glauca (Moench) Voss) OP families represented by 1,694 individuals growing on one site in Quebec, Canada. Assuming half-sibling, the pedigree-based model was limited to estimating the additive genetic variances which, in turn, were grossly overestimated as they were confounded by very minor dominance and major additive-by-additive epistatic genetic variances. In contrast, the implemented genomic pairwise realized relationship models allowed the disentanglement of additive from all non-additive factors through genetic variance decomposition. The marker-based models produced more realistic narrow-sense heritability estimates and, for the first time, allowed estimating the dominance and epistatic genetic variances from OP testing. In addition, the genomic models showed better prediction accuracies compared to pedigree models and were able to predict individual breeding values for new individuals from untested families, which was not possible using the pedigree based model.Clearly, the use of marker-based relationship approach is effective in estimating the quantitative genetic parameters of complex traits' even under simple and shallow pedigree structure.

Spatial and competition effects in tree breeding

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Local environmental variation is well known to bias genetic estimates if not accounted for properly.Several authors have recommended to routinely include spatial effects in models for genetic evaluation of trees [e.g. @Gilmour97; @Dutkowski02].In contrast, the competition among trees is a known issue but much less frequently addressed and studied [@Muir05; @Cappa08; @Costa13].It produces a negative autocorrelation among neighbouring trees, which can compensate in part the spatial effect, making both effects more difficult to detect and separate.Moreover, it can have an important impact in the response to selection if not accounted for.First, because of biased genetic estimates. But most importantly, because direct and competition additive genetic effects are often antagonistic.Hence, selecting the *best* genotypes frequently means also selecting the most competitive individuals, which is not necessarily the optimal strategy.In addition, any phenotypic measure could potentially benefit from spatial and competition adjustments, delivering records that are less prone to bias by uncontrolled or hidden environmental factors, and therefore with clearer genetic signal for further use in association and genomic predictions.In this talk, we illustrate the advantages in the use of spatial and competition evaluation models through a Douglas-fir case study from the french breeding program.We also discuss diagnostic tools and procedures, as well as current implementations of spatial and competition models available in the Free and Open Source Software R-package `breedR`.

Alternative approaches to modeling breeding value in tree breeding programs

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The goal of this study is to improve selection methods in tree breeding programs, by using patterns of gene expression and genetic variation in coding sequences to model parental breeding values. We hypothesize that early selection can be based on data from RNA-seq experiments on two conditions – first, that there are genetic differences among parent trees in gene regulatory networks, and second, that those differences are correlated with family mean performance in progeny field tests. Testing these conditions requires (1) obtaining reproducible estimates of gene expression from replicated samples of seedlings from OP, PMX, or CP families; (2) combining those family-mean estimates of gene expression levels into covariance estimates for parents that show predictive power in cross-validation studies for modeling phenotypic variation, and (3) combining covariance matrices based on coding sequence SNP variation, gene expression level variation, and pedigree-based estimates of allele sharing to optimize predictive modeling of phenotypic variation. A preliminary study is underway with a total of 62 different parent trees of Pinus taeda (loblolly pine), from a wide geographic distribution, with existing progeny field test data available from multiple sites. Seedlots (OP/PMX in 54 cases, CP in 8 cases) from these parents were grown in two different batches in a greenhouse, and pooled seedlings were harvested at age 3 months for RNA extraction, cDNA library preparation, and high-throughput sequencing. The reproducibility of family-mean gene expression patterns and the extent of differential gene expression have been assessed, and covariance matrices of gene expression and SNP variation are being used to model phenotypic variation in progeny field test data.

Biomass growth rate and effects of eCO2 – some simple theory and preliminary observations in Eucalypts

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There is interest in both characterising and estimating the effects of elevated carbon dioxide concentration (eCO2) on plant growth. In general we have more skill in describing photosynthesis than in describing the partitioning of the photosynthate to leaves versus the rest of the plant. This is true of many perturbations in addition to eCO--2. We here attempt to address some of this deficit.

CLOSING KEYNOTE LECTURE: **Antoine Kremer**

Antoine Kremer
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Concluding remarks: "Assisted migration into the wild"

Poster Abstracts

SESSION 1

Poster number : S1.1

Adaptive species divergence in oaks and the identification of candidate genes in species with whole genome sequences (oaks and poplar)

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The ecologically divergent but hybridizing oak species, Quercus rubra and Quercus ellipsoidalis, provide a model for the discovery of genes that are involved in reproductive isolation and adaptive divergence between these species. Scoring of nuclear Simple Sequence Repeats (nSSRs) and of genic EST-SSRs in neighboring interspecific population pairs revealed a set of candidate genes for drought tolerance and phenology with very high interspecific differentiation as signatures of divergent selection (outlier loci). Thus the trinucleotide microsatellite FIR013 was nearly fixed on alternate alleles in the drought tolerant Q. ellipsoidalis and in the drought averse Q. rubra and was identified as under very strong divergent selection in four interspecific population pairs (Lind-Riehl et al. 2014). The trinucleotide microsatellite is located in the coding region of a CONSTANS-like gene (COL) and encodes for poly (E) repeat. The Q. ellipsoidalis allele (138bp) differs from the Q. rubra allele (141 bp) by one glutamine residue. In other studies, nucleotide variation in the same COL gene was associated with spring phenology in Quercus petraea (Alberto et al. 2013) and variation in poly (E) repeat length in COL2B was associated with growth cessation in Populus tremuloides (Ma et al. 2010). With the availability of a whole genome sequence in Q. robur (Plomion et al. 2016), genome-wide outlier screens and construction of chromosome anchored high density linkage maps in oaks can be used to map the genomic distribution of outlier genomic regions and underlying genes, and their co-localization with Quantitative Trait Loci (QTL) for adaptive species differences. For example, using a genome-anchored linkage map in poplar, QTL analyses of leaf phenotypes and gene expression identified a major QTL and a prime candidate for leaf shape differences between Populus deltoides and Populus trichocarpa (Drost et al. 2015). AcknowledgementsFunding for the research on oaks (# 1025974) and poplar (# 1230803) was provided by the National Science Foundation. Additional support was provided by the Hanes Trust.

Poster number : S1.2

Genetic variability of common beech (Fagus sylvatica L.) in a provenance trial "Medvednica"

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International beech provenance trial Medvednica is located on the homonymous mountain near Zagreb, Croatia. Trial was established in 2007 with 21 provenances from 9 European countries. The trial was laid out according to RCBD experimental design, with each provenance represented by 50 plants in three replications (blocks), planted in rectangular plots with 2.0 × 1.0 m spacing (Ivanković et al. 2011). The aim of this study was to determine the amount and pattern of adaptive genetic variability among populations for height growth, survival and flushing phenology. Analyses of variance (ANOVA) were performed using the MIXED procedure in SAS software. Results showed significant differentiation among provenances in flushing phenology and height. Between provenance differences were further tested with post-hoc Tukey–Kramer's test which showed higher level of differentiation for flushing phenology and lower level of differentiation for height growth. Multivariate regression tree

(MRT) analysis was used to determine the pattern of genetic differentiation (Hamann et al. 2011). MRT analysis of heights and survival rates separated provenances with respect to the latitude and longitude. Provenances from eastern habitats had lower mean heights and mean survival rate than those originating from the western habitats. MRT analysis of flushing phenology separated provenances with respect to the continentality variable and humidity. Provenances originating from relatively more humid habitats flushed later. With the increase of continentality and humidity, provenances showed a trend of earlier flushing. Results of this study suggest genetic differences among provenances, driven by natural selection in original sites. Further research in this provenance trial will provide better insight about adaptability of the provenances in a given environmental conditions. According to Gömöry (2009), researches like this are important for identification of provenances which are characterized by good growth and adaptability, in order to use it as a seed source for future reforestations.

Poster number : S1.3

Single-locus versus Multilocus Patterns of Genetic Architecture Underlying Local Adaptation to Climate in a Wide-Ranging Species, Eastern White Pine (Pinus strobus, Pinaceae)

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Natural plant populations are often adapted to their local climate and environmental conditions, and populations of forest trees offer some of the best examples of this pattern. However, little empirical work has focused on the relative contribution of single-locus versus multilocus effects to the genetic architecture of local adaptation in plants/forest trees. Here, we employ eastern white pine (Pinus strobus) to test the hypothesis that it is the effects among loci that primarily drive climate-induced local adaptation. The genetic structure of 29 range-wide natural populations of eastern white pine was determined in relation to local climatic factors using both a reference set of SSR markers, and SNPs located in candidate genes putatively involved in adaptive response to climate. Comparisons were made between marker sets using standard single-locus outlier analysis, single-locus and multilocus environment association analyses and a novel implementation of Population Graphs. Magnitudes of population structure were similar between the two marker sets. Outlier loci consistent with diversifying selection were rare for both SNPs and SSRs. However, genetic distances based on the multilocus among population covariances (cGD) were significantly more correlated to climate, even after correcting for spatial effects, for SNPs as compared to SSRs. Coalescent simulations confirmed that the differences in mutation rates between SSRs and SNPs did not affect the topologies of the Population Graphs, and hence values of cGD and their correlations with associated climate variables. We conclude that the multilocus covariances among populations primarily reflect adaptation to local climate and environment in eastern white pine. This result highlights the complexity of the genetic architecture of adaptive traits, as well as the need to consider multilocus effects in studies of local adaptation.

Poster number : S1.4

Detecting selection by climate in Mexican populations of Quercus rugosa

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Local adaptation is a key factor shaping the traits that underlie the growth and survival of tree species and contributing to genetic differences among populations. Here, we test whether spatially divergent selection is acting to generate locally adaptive genetic variation among high-elevation populationsof a widespread Mexican oak (Quercus rugosa). Using 6873 random single-nucleotide polymorphisms (SNPs) genotyped from 105 individuals across Q. rugosa populations in the Trans-Mexican Volcanic Belt (TMVB), we tested for genes associated with local adaptation by identifying SNPs that show high population divergence using BayeScan and SNPs that are associated with climate gradients using Latent Factor Mixed Models (LFMM). Genetic structure and multivariate analyses were performed to provide context for interpreting patterns of selection. Partial redundancy analysis indicates that both geographic structure and climate influence genetic variation. Climate predictors explain 53.2% and spatial predictors explain 42.9% of total genetic variance. We found 96 outlier FST SNPs, 108 SNPs that are significantly associated with climate, and one SNP is common among these sets. However, most of the climate-associated SNPs are not located at the 5% upper tail of the FST distribution. The genomic contexts of the 203 candidate SNPs indicated that 138 SNPs were predicted to fall within 115 genes, while 61 SNPs neighbored 51 unique genes. We identified proteins involved in a broad range of biological processes, and nine of these proteins are directly involved in response to abiotic and biotic stimuli, such as temperature stress, response to radiation, oxidative stress, response to chemical stimulus, detoxification and response against bacteria. Our results provide a snapshot of the role of geography and climate in shaping genetic variation of Q. rugosa. This study also contributes to our understanding of the mechanisms responsible for the local adaptation to climate in forest trees. ACKNOWLEDGEMENTSThis research was supported by a UC-MEXUS-CONACyT collaborative grant to AG-R and VLS, and UCLA seed funding to VLS. Illumina sequencing was performed at UCLA Broad Stem Cell Research Center. We used computational and storage services associated with the Hoffman2 Shared Cluster provided by UCLA. KM received a PDE scholarship from “Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil”.

Poster number : S1.5

Geographic clines and adaptation in germination, growth and survival features of Pinus densiflora seedlings in the common-garden experiment in Japan

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Pinus densiflora (Japanese red pine) is both an economically and ecologically important conifer, which constitutes one of major forest landscapes in semi-mountainous area in Japan. Since this species is suffering from serious reduction in population sizes throughout Japan because of recent northern expansion of pine wilt disease, adequate program design to conserve or manage its regional genetic resources and breeding materials is essential. Recent molecular studies have evaluated levels of current genetic diversity and structure of populations for most of major tree species in Japan (e.g., Takahashi et al. 2005 for Cryptomeria japonica; Hiraoka and Tomaru 2009 for Fagus crenata) as well as P. densiflora (Iwaizumi et al. 2013). However, despite of diverse climatic and environmental conditions of Japan archipelago, almost no information about phenology and morphology has been obtained for Japanese forest tree species on the levels of adaptive genetic variation and the potential of adaptation or plasticity against climatic changes. Under such circumstances, in 2013, Forest Tree Breeding Center (FTBC) has started common-garden experiment firstly for P. densiflora with a systematic design. The same sets of 50 maternal families derived from 10 provenances covering the species’ natural distribution were seeded (or are to be seeded) at 5 to 6 different sites of FTBC throughout Japan. In the present study, we examined the geographic patterns on the variation in germination, growth and surviving traits during current-year and 2-year seedlings at the site Shoo, Okayama, mid-western Japan (35.06°N, 134.11°E).We found that the germination rate of current-year seedlings was significantly greater in northern families, which germination period started and ended

earlier than southern families. The relative growth rate (RGR) of 2-year seedlings was greater in northern families, which shoot elongation period also started and ended earlier than southern families. The surviving rate was low in most of families of three provenances which were southward from Shoo.Earlier germination and growth phenology in northern provenances indicate the lower cumulative temperature to start, possibly related to the adaptation to lower temperature. The lower surviving rate in southern families is corresponding to the previous study of pine seed transfer that suggests low performance of southern seed origin in northern plantation (Nagamitsu et al. 2015). In near future investigations at different sites enable us to examine climatic responses in several adaptive features such as growth, reproduction and the physiological backgrounds, which is fundamental information to consider ex-situ conservation unit or breeding zones.

Poster number : S1.6

Genetic architecture of reproductive isolation and species differences in Populus alba and P. tremula

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Research in speciation genomics has recently received great benefit from advancements in genomic technologies and from the introduction of refined models describing the evolutionary processes generating biological diversity. Currently, a field attracting much attention is the study of “divergence-with-gene-flow”, i.e. divergence involving episodes of sympatry or parapatry and opportunities for genetic contact, before reproductive isolation between incipient species is complete. In this project, we address key questions related to the ecological and evolutionary genomics of “divergence-with-gene-flow” in Populus alba and P. tremula, two widespread Eurasian tree species still able to hybridize despite great genetic and ecological divergence. In particular, the project focuses on (1) investigating the reproductive barriers between the two species, and (2) unraveling the genetic architecture of ecological differences maintained in the face of gene flow. These topics are addressed with the help of Restriction site Associated DNA sequencing (RAD-seq) of common garden grown seedling families, and by coupling genomics with evolutionary ecology experiments. Towards addressing objective (1), we examined the relationship between genomic ancestry and survivorship in hybrid seedlings growing in a common garden trial. The results indicate higher mortality of genetically intermediate recombinant hybrids compared to first generation hybrids (F1), likely explaining the predominance of F1 among adult trees in natural hybrid zones. This observation suggests that selection is acting to maintain strong postzygotic barriers between the two species and speaks for a role of intragenomic coadaptation of loci in hybrid breakdown. Early post-mating reproductive isolation and the genomic regions contributing to it will be further studied by examining patterns of segregation distortion in the progeny of a controlled cross. To facilitate characterizing the genetic architecture of species differences (objective 2), we genotyped hybrid seedlings from two common garden locations and measured their phenotypes for numerous ecologically important traits. Local patterns of genomic ancestry revealed the recombinant nature of the majority of the seedlings, thus allowing the use of admixture mapping methodology to link genomic and phenotypic information to infer the chromosomal position of quantitative trait loci responsible for the traits. In addition, individuals with potentially transgressive phenotypes were identified, and these will be investigated further to determine their potential role in weakening the reproductive barriers between these two important foundation

Poster number : S1.7

Genetic mapping of Gene Copy Number Variations in White Spruce (Picea glauca) and QTL analyses for quantitative adaptive traits

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Several genes in copy number variation (CNV) have been reported in model organisms for their involvement in phenotypic variation such as starch metabolism in human [1] or pathogen resistance in soybean [2], for instance. Using comparative genomic hybridization on arrays (aCGH) targeting more than 14,000 genes, we previously identified 2,443 genes in CNV in white spruce (Picea glauca; manuscript in prep). The objectives of the present work were to map a maximum of those genes upon the genetic map of white spruce and test whether these CNVs have an impact upon quantitative trait variations in this species.We designed a new CGH array targeting those genes in CNV to efficiently test more than 100 individuals for each of two white spruce families from eastern Canada already studied for genetic mapping and QTL mapping for adaptive traits and growth [3]. Each descendant was compared to the same reference parent within each family and self-self comparisons of the reference genomes were used to evaluate the false discovery rate. Additional comparisons between parents and haploid samples allowed to infer the heterozygous status of parents for each gene in CNV, i.e. same or different numbers of copies on both chromosomes. Based upon the most recent published genetic map [4] mostly encompassing gene SNPs, a pseudo-test cross approach was then applied to successfully map the subset of CNVs that present adjacent copies. To date, the analysis of the first family allowed to map dozens of these CNVs that were spread over 10 of the 12 linkage groups. Clustering of CNVs was observed to form putative CNV hotspots.This successful mapping of CNVs is a first step towards a de novo QTL mapping analysis for adaptive traits and growth using CNVs as additional genetic markers and published phenotypic data [3]. Comparing the genetic map enriched with CNVs and the reported QTL mapping results [3] revealed 24 mapped genes in CNV that were included in genomic regions involved in adaptive quantitative trait variations in white spruce.To our knowledge, this work aiming at localizing CNVs upon a genetic map by means of aCGH testing wide full-sib families represents an original approach that paves the way for many other model and non-model species.

Poster number : S1.8

Reproductive success and species occupancy changes during natural regeneration in a mixed oak stand (Quercus petraea and Quercus robur).

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A genetic survey was conducted within two successive generations in a mixed Quercus petraea -Q. robur stand with the aim to assess reproductive success and changes of the species occupancy after natural regeneration. The stand-that was 95 years old- was managed as an even aged stand and a seed cut was implemented leaving 260 seed trees, which will subsequently be called the “parental cohort”. In 2000 complete natural regeneration was achieved, and all seed trees were cut. In 2014, we sampled systematically 2490 seedlings within the natural regeneration (“offspring cohort”). All trees of the two cohorts were genotyped with 126 SNPs (four multiplexed assays) using a Sequenom® MassARRAY® MALDI-TOF MS platform and iPLEX™ Gold chemistry. After quality checking, and visual assessments of the scatter plots, 82 SNPs were finally maintained for the genetic analysis. All trees of the parental and offspring cohorts were assigned to one of the two species (or hybrids) according to admixture values obtained by STRUCTURE analysis.Among the 260 trees of the parental cohort, 135 were assigned to Q. robur (52%), 109 to Q. petraea (42%) and 16 to admixed individuals (6%). Corresponding figures within the 2490 seedlings of the offspring cohort were: 811 Q. robur (33%), 1548 Q. petraea (62%) and 131 admixed individuals (5%). These results indicate a shift of the taxonomic composition of the stand and are further confirmed by the comparison of the spatial

distribution of the two species along the two generation. Q. petraea tends to extend its distribution. A parentage analysis was subsequently conducted where parents were tentatively assigned to of each offspring using CERVUS. For 1285 seedlings (51.6%) among the 2487 that could be successively genotyped, at least one parent could be retrieved. For 329 of the seedlings among the 1285, the two parents could be successively assigned, and for 956 seedlings only one parent could be assigned. Among the 260 trees of the parental cohort only 24 were not assigned as parent to any of the seedlings. Reproductive success of each parent trees was further inferred from the parentage analysis.There is an uneven distribution of reproductive success in both species: while a few trees produce a large amount of offspring, most trees show low reproductive success. Mean reproductive success is significantly higher in Q. petraea (7.4) than in Q. robur (5.6), and thus confirms the earlier reported demographic shift. These data will ultimately be used to calculate selection gradients and monitor evolutionary changes that occur over two successive generations in oak forests.

Poster number : S1.9

Genetic variability analysis and conservation status of Terminalia arjuna L. (Arjun) in Achanakmar Amarkantak Biosphere Reserve (AABR), Chhattisgarh, India

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Achanakmar Amarkantak Biosphere Reserve, Chhattisgarh, India is a biodiversity hub of Central India. Terminalia arjuna is one of the potential medicinal tree of this Biosphere Reserve. In the present investigation an attempt has been made to assess the genetic diversity of T. arjuna and sixteen accessions of T.arjuna of diverse geographical locations from AABR were selected. Random Amplified Polymorphic DNA(RAPD) markers were used to evaluate the genetic diversity of T. arjuna. The six Rbac primers were used for the RAPD analysis. In the present study a total of 515 bands yielded from 6 reproducible primers, with a mean of 85.8 amplified bands per primer. The Rbac 1 primer showed maximum polymorphism (31.5%) and Rbic 5 showed lowest polymorphism (0.025%). This forest tree species showed 47% to 92% similarity at genetic level in different clusters. A dendrogram clustered, based on the UPMGA clustering method revealed three clusters(A,B and C). Cluster A has maximum number of genotypes (12) and rest two have only two-two genotypes. Cluster A was further divided in five sub-clusters. The genotypes in A clusters had highest diversity while genotypes fall in cluster B and C clusters had lowest genetic diversity. The results of present investigation showed that T. arjuna possess high level of genetic diversity within the biosphere reserve. This study is the first investigation on molecular characterization of T. arjuna in the Achanakmar Amarkantak Biosphere Reserve, which will help to layout the framework for its genotype conservation in situ for AABR.

Poster number : S1.10

Genetic diversity of Sweet chestnut (Castanea sativa Mill.) populations of Central and South-eastern European origin.

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Sweet chestnut (Castanea sativa Mill.), is a widely distributed European forest tree species of great economic importance, valued not only for its fruit and timber, but also for its contribution to the landscape and the environment. Research conducted in the Mediterranean basin and some north-western European populations with the use of molecular markers indicate five distinct C. sativa gene pools, three of which are located in Greece.Eleven populations from Greece, Germany, Italy, and Bosnia and Herzegovina were analysed using chloroplast (cpSSRs) and nuclear (nSSRs) molecular markers. Between 16 and 25 samples were analysed per population. All six tested chloroplast microsatellite markers were polymorphic. Haploid diversity varied between 0.036 (Kostajnica-Bosnia) and 0.422 (Hortiatis-Greece). The results reveal a notable genetic differentiation of the Greek

populations compared to the other studied C. sativa populations. All seven tested nuclear microsatellite markers were polymorphic, but with a higher level of polymorphism as compared to the cpSSR markers. A total of 48 different alleles were identified in 215 individuals and the number of detected alleles for each locus varied between 4 and 15, with a mean of 6.85 alleles per locus.Statistical analysis using STRUCTRE software revealed five clusters. One cluster was formed by the Greek populations, another cluster by the German populations. The Bosnian populations represent two groups consisting of two and four populations. The Italian population appears to form a separate cluster sharing some genetic information with two Bosnian populations.

Poster number : S1.11

Pinus sylvestris population structure studied with exome sequencing

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Studies of population structure throughout the distribution range of Pinus sylvestris have so far revealed very minor differences between populations. Detecting the putative underlying finer structure demands considerably larger datasets. As recent development of genomic tools in laboratory protocols and bioinformatics are finally making targeted sequencing possible in conifers, we have developed a set of 60 000 baits for targeted sequencing of over 3000 genes in P. sylvestris. We have also developed a custom bioinformatics software to automatize and parallelize the analysis of samples in supercomputing environment. The workflow has been designed to minimize the issues stemming from the massive genome which contains considerable amount of paralogous sequence. About 150 samples throughout the P. sylvestris distribution have been sequenced with Illumina 2500 instrument to produce the largest set of SNPs for this species to date. We analyze the population structure with PCA, STRUCTURE, F statistics and patterns of linkage disequilibrium. In future the sampling scheme allows us to recognize genomic regions contributing to local adaptation by searching for correlations between clinal environmental variables and allele frequencies.

SESSION 2

Poster number : S2.1

Environmental heterogeneity and phenotypic variance in Pinus sylvestris, Pinus halepensis and Pinus pinaster

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There is a need to understand what the relationship among genetic diversity and environmental heterogeneity in forest trees is, in order to elucidate the adaptation mechanism in long lived organisms like trees. The aim of this study was investigate the relationship between environmental heterogeneity and phenotypic variation in populations of Pinus sylvestris, P. halepensis, and P. pinaster. We used data in a set of provenance trials located in Spain. We measured diameter at breast height (mm), total height (cm), survival (presence/absence), male and female flowering (number of catkins/cones, only in P. halepensis) at the age of 14-17 year old. We estimated using mixed models the mean and variance of each population and a plasticity index (Valladares et al., 2006) from the different sites. We measured environmental heterogeneity from each population at different spatial scales (5, 10, 50, 100 and 200 km2) using climatic variables and we selected those had a response in phenotypic variables. We adjusted linear models to establish the relationship between phenotypic variability and environmental heterogeneity. The models were selected based on Akaike's parsimonious criterion (Burnham & Anderson, 2002). We found that at 10, 50 and 100 km2 scales had a strong influence of climatic variables was detected for some of the phenotypic variation. We obtained the significant models for P. sylvestris, P. halepensis and P. pinaster. All of these models had a good Level of

Empirical Support of Model (Di <2). Finally we found the climatic variables associated with phenotypic variables in every species.

Poster number : S2.2

On the hunt for drought resistance markers in Norway spruce

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Climate change and attendant extreme climatic events turned out to become 'an inconvenient truth'. Especially heat waves have dramatic consequences, as the European heat wave of 2003 contributed to a 30 % reduction in gross primary production of terrestrial ecosystems over Europe [1]. Forests are particularly sensitive to climate change, because the long life-span of trees does not allow for rapid adaptation to environmental changes. In order to speed up breeding and selection strategies to improve a tree species' resilience against drought, the hunt for respective markers has begun.By using Norway spruce (Picea abies (L.) H.Karst.) as the organism of choice – since it is one of the most economically important coniferous species in Europe – we followed two strategies for the discovery of drought stress associated molecular markers. At first, the analysis of two phenotypic pools (drought resistant versus sensitive) by applying the MRE-seq strategy [2] led us to the promising extraction of 173 SNPs. Selected putative markers were re-sequenced on a wider set of samples, however, an association to drought resistance could not be confirmed. Possible pitfalls causing the failure of this attempt will be discussed. In the second approach, seedlings of 130 accessions from 5 European countries were phenotyped regarding their drought resistance in a common garden experiment. 211 samples of 35 chosen accessions were genotyped on a SNP chip comprising 3.257 sites. Phenotype-genotype correlation analysis using the general linear model (GLM) implemented in Tassel [3] revealed 6 markers significantly correlated with at least one of the defined traits ($p < 0,0002$ after Bonferroni correction). Five markers are associated to genes and are all located downstream in a range from 1 – 651 bp. Besides a gene with bZIP transcription factor activity, genes involved in binding functions and glucose catabolic processes were identified.These results provide a valuable knowledgebase for facilitating our understanding of drought stress response in Norway spruce. Furthermore, the herein described putative markers might accelerate genetic improvement through marker-assisted selection in future.Acknowledgments: This study was kindly supported by the Austrian Research Promotion Agency (#834209), Forstbetrieb Mayr-Melnhof, Kooperationsplattform Forst Holz Papier, Lieco, and Österreichische Bundesforste.

Poster number : S2.3

Clinal variation in FLT2 and GI at high latitudes in Siberian spruce

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Intraspecific latitudinal clines in genotypes and phenotypes are widely interpreted as evidence for local adaptation. Parallel latitudinal clines in gene expression and allele frequency at FTL2 and Gigantea (GI), two genes from the photoperiodic and the circadian clock, respectively, were observed in both Norway spruce (Picea abies) and Siberian spruce (Picea obovata). Transformation of FTL2 in Norway spruce also demonstrated that high expression levels lead to early budset. In the present study we analyzed clinal variation at FTL2, GI and 14 background genes from seven P. obovata populations located along the Ob River in Siberia, from latitude 61°N to 67°N. The steepest change in phenology occurs between these latitudes and they were under-represented in our previous studies. We also sequenced around 12,000 bp of GI, a much larger part of the gene than done earlier on. As in previous studies clinal variation in growth cessation was strong. Allele frequencies in both FTL2 and GI were correlated with latitude, and a Bayenv analysis showed a significant enrichment for SNPs from candidate genes. However, only FTL2 showed a significant cline in expression with latitude.

Finally, we did not detect any departure from the standard neutral model in GI. Overall, the present results lend further support to the importance of variation at FLT2 and GI for local adaptation in tree species.

Poster number : S2.4

Inferring selection during long range colonisation: the Aleppo pine (Pinus halepensis) in the Mediterranean Basin

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The Aleppo pine (Pinus halepensis Mill) has a circum-Mediterranean distribution that is both geographically fragmented and large, spanning 3.5 million hectares. The demographic history of Aleppo pine is characterised by westward range expansions from the eastern Mediterranean Basin. This pine lives nowadays in a wide range of environmental conditions, which reflects a high degree of adaptability over large range expansions. Identifying alleles linked to environmental variables is challenging in such context, as differences in allelic frequencies may reflect signals of selection or translate gene surfing, i.e. the spread of neutral mutations in an expanding population front. In this study, we use an unprecedented number of molecular markers as well as populations, to better understand the colonisation history of Aleppo pine and how it has adapted to its environment at the molecular level. We present a framework by which to detect footprints of selection while correcting for population structure in expanding populations, as well as exploring if long range colonisation events can yield false positives due to the stochastic action of gene surfing, using both real and simulated data. We find that the Aleppo pine shows a previously unsuspected genetic structure across its range, as well as evidence of SNPs under selection in response to environmental variables such as precipitation and temperature, which could give us a clue as to why this pine has managed to colonise such a vast range.

Poster number : S2.5

Genetic differentitation of pedunculate oak (Quercus robur L.) provenances along a latitude gradient in responses to drought and rewatering

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Acorns were collected in 2013 in nine Quercus robur L. populations along a latitudinal gradient from Estonia to Italy. Seedlings were raised during 2014 in a nursery and individually transplanted in 50l plastic pots with homogenised soil from a local oak forest. A greenhouse trial was established in spring 2015 according to RCBD split plot design with two main plots. Plants in the first plot did not receive any water from April 1st till July 21st (drought treatment), while plants in the second plot (control) were constantly kept at 45-50% of soil moisture content (SMC). Plants in the drought plot were rewatered on July 21st and kept at 45-50% of SMC till the end of the growing season. Heights and photosynthetic rates (PhRs) were periodically measured on all plants. The aim of the study was determining phenotypic responses of the provenances to drought and rewatering and assessing amount and pattern of among provenance genetic variation (Bogdan et al 2015ab, Sever et al 2015).Repeated measures ANOVA were performed using proc MIXED of the SAS software. Effects of

time, treatment, provenance and their interactions were determined. Statistical significance of differences between various effect levels was determined by Post-hoc Tukey test. Multivariate regression tree (MRT) analyses using MVpart procedure in R were conducted to reveal a pattern of genetic differentiation among the provenances (De'Ath et al 2002). MRT were done combining phenotypic data from the trial and mean annual climate data of the provenance original sites from the ClimateEU software (Wang et al 2012, Hamann et al 2011).Height growth of all provenances decreased as response to drought. Mean heights of most provenances were more than 50% lower, compared to the control on the peak of drought. However, some provenances showed milder decrease (e.g. Hungarian, Lithuanian and Estonian, whose growth decreased 35, 40 and 4%, respectively). Mean PhR of provenances in the drought treatment was also significantly lower, compared to control. For both traits, between provenance variations were highly significant ($p<0.001$). After rewatering, all provenances showed rapid recovery in both traits. Provenance mean PhRs in drought treatment outperformed those in the control, just two days after rewatering. Variation among provenances in the speed of recovery, as well as in final recovery was also statistically significant. MRT analysis clustered provenances according to mean growing season precipitation at their original sites (which explained 62% of the variation).

Poster number : S2.6

Phenotypic differentiation of native and introduced populations of Northern Red Oak (Quercus rubra L.)

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Native to North America, Northern red oak (Quercus rubra) was introduced in Europe in the XVIIth century for ornamental and forestry purposes. This species is now widespread in European forests due to plantations and natural regeneration. In invasive herbaceous plants, introduced populations are often genetically different from native populations. However, this has been poorly investigated in exotic tree species. Our objective was to explore the phenotypic variation between native and introduced populations of Q. rubra and to test for adaptation to the new environmental conditions since the introduction.We used three progeny test gardens, in South-Western, Central and North-Eastern France, composed of 64 American and 42 European populations. The gardens were settled from 1980s and trees were monitored regularly for growth (diameter, height) and leaf phenology (budburst, coloration). For two years, we have monitored acorn production. Within each garden, data were analyzed using mixed analyses of variance; Qst indexes were calculated to evaluate genetic differentiation between populations.Overall, introduced populations presented higher trait values than native populations: growth rate was higher and spring phenology was advanced. Fruit set was higher in introduced trees, although depending of the year. Qst estimates clearly demonstrated the existence of a high genetic differentiation between native populations, for growth and phenology. Introduced populations presented a lower level of differentiation, significant for phenology, but not for growth.These results suggest several hypotheses: (i) introduced populations only represent a part of the global diversity existing in the native range (ii) populations have evolved since introduction under new environmental selective pressures (ii) populations were selected by man since introduction. These hypotheses are being investigated, notably through a molecular approach.

Poster number : S2.7

CO2-response QTL for leaf gas exchange and water use efficiency in Quercus robur

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Intrinsic water use efficiency (Wi) is the ratio between net leaf CO2 assimilation rate (A) and stomatal conductance to water vapour (gs). QTL have been detected for A, gs or Wi, mainly for crop species (rice, what, maize), Arabidopsis and a few tree species (poplar, apple, maritime pine and pedonculate oak). However the large number of individuals to measure usual for QTL studies complicates the estimation of genotype-typical values by introducing response-variations during the measurement campaign. Repeated measurements and multi-environment QTL analyses are one approach to gain detection power. In this study, we investigated the impact of elevated atmospheric CO2 concentration on diversity of A, gs and Wi in a Quercus robur mapping population. The atmospheric CO2 concentration impacts both, A and gs. Short term responses to an increased atmospheric CO2 concentration are an increase in A and a decrease in gs, resulting in an increased Wi. However, plant co-regulate A and gs, resulting in a complex system. For long-term exposure to elevated CO2, acclimation can also play a role and impact A and gs differently, which would have a large impact on the temporal evolution of Wi. 183 three-year old cuttings from a Quercus robur full-sib family were exposed before bud flushing in two adjacent greenhouses to two different CO2 levels (ambient, controlled : 380 µmolmol-1 and elevated : 690 µmolmol-1). Gas exchange measurements were done on five dates from May until September. Combining 5 dates with two CO2 levels, resulted in 10 data sets that were combined in one QTL analysis similarly to a full-cross statistical analysis. The multi-environment QTL analysis (MultiQTL) was used to test for significant date and/or CO2 differences for the allelic effects of the detected QTL, where significance is an indication for a plasticity QTL. Plasticity QTL were also detected for the CO2 effect by using the difference between environments for A, gs and Wi as an estimator of plasticity. A significant CO2 or CO2 x date effect has been detected for two QTL for gs, whereas a significant date effect has been detected for two QTL for A and Wi, respectively. The results will be discussed in terms of allelic sensitivity or gene regulation plasticity theory. The detected QTL for Wi will play an important role in the ongoing French national project H2Oak (https://www6.inra.fr/anr-h2oak/) to select positional candidate genes for screening in natural populations.

Poster number : S2.8

Genetic mapping of resistance to root rot disease (Phytophthora cinnamomi) for American chestnut restoration in the southeastern United States

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The soil-born oomycete Phytophthora cinnamomi (Pc) causing root rot and the ascomycete fungus Cryphonectria parasitica (Cp) causing chestnut blight are two major pathogens of the American chestnut Castaneae dentata. Due to favorable climatic conditions for its life cycle, Pc has an especially severe impact on chestnut stands in the southern US. To address a need for pyramiding resistance to both pathogens, a collaboration among the USDA-Forest Service, The Forest Health Research and Education Center, The American Chestnut Foundation, Clemson University and The Chestnut Return Farm was established for screening Cp-resistant hybrid material from the TACF breeding program for

resistance to Pc. In parallel, a study was initiated to determine the genetic basis for Pc resistance and develop markers to identify and track Pc resistance in advanced breeding material.Using the backcross families derived from crosses of American chestnuts with two resistant Chinese chestnut trees, 'Mahogany' and 'Nanking', we conducted linkage mapping and QTL detection for the Pc resistance introgressed from Chinese chestnut. Utilizing a genome-wide SNP array, an initial low density genetic map was constructed for a limited number of individuals issued from a cross of AdairKY1 × GL158 (Nanking background). Resistance to Pc was mapped to linkage group E (LG_E). Using a set of the LG_E SSR markers from the Chinese chestnut reference map, we also genotyped additional hybrid populations and generated local LG_E maps for half sib crosses NK1+NK2 (Nanking background) and KY115 × AD98 (Mahogany background). Overlapping QTL for Pc resistance were detected in both Chinese chestnut Pc resistance sources. For a more comprehensive QTL study, we generated extended hybrid populations and phenotyped those for resistance to Pc. Altogether, 1408 phenotyped individuals (5 crosses) are now available and are currently being mapped using "Genotype By Sequencing" (GBS) platforms. We have currently completed a GBS map and QTL analysis of one of these crosses (HB2 family) that carries resistance donated by the Chinese chestnut tree 'Mahogany'. Here we present the results of this collaborative research effort and discuss their implications for breeding Pc resistant American chestnut.

Poster number : S2.9

Quantitative and molecular genetic variation in closely related European pine species.

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Ecologically differentiated but recently diverged species can be good systems for studying the genomic signatures of selection. We studied genetic variation in phenology and growth in a common garden trial, and patterns of nucleotide polymorphism and divergence in candidate nuclear genes and the whole transcriptome in four closely related pine species: Pinus sylvestris, P. mugo, P. uliginosa and P. uncinata. Differences were found in quantitative traits both among and within species. Patterns of phenotypic variation indicate that, during speciation, selection has operated on different traits according to the specific environment occupied by each species. All species shared a common genetic background, against which we identified several non-neutral loci, which showed intraspecific differentiation but also interspecific divergence. The results suggest that selection has operated at these loci on both evolutionary and ecological timescales. Transcriptomic data provided a large set of SNPs across species (~220-262k) for any future population and association genetic studies in pines.

Poster number : S2.10

Genetic analysis of oleoresin terpenoid content in maritime pine

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Oleoresin terpenoids are major volatile and non-volatile defense compounds of conifers. Much is known about the biosynthesis of conifer terpenoids on the level of enzyme biochemistry, molecular biology and genome annotation, in particular for the isoprenyl transferases, terpene synthases and cytochrome P450s that catalyze the formation of a large diversity of conifer terpenoid structures. By comparison, less is known about the genetics of terpene biosynthesis in conifers.In the present study, 18 monoterpenes (C10) and sesquiterpenes (C15) were quantified in two maritime pine trials. The first consisted on 300 five-year old genotypes collected in a provenance-progeny trial, covering four genetically distinct populations. This sampling design enabled us to estimate heritability and infer evolutionary forces acting on these compounds. The second included 180 fifteen-year old genotypes from a full-sib pedigree and allowed to detect genomic regions (QTLs) involved in phenotypic variability.Medium to high heritabilities were found for the studied terpenes suggesting a strong genetic control for these compounds. By comparing neutral genetic (FST) and phenotypic (QST) differentiation among populations, cases of positive selection were identified (e.g. for β-pinene). Besides, a total of

22 QTLs corresponding to 6 different regions were detected. Two regions, each gathering 6 QTLs, were found to be specific to monoterpene or sesquiterpene, suggesting a pleiotropic effect or physically linked genes involved in the biosynthesis of these two groups of terpenes. The percentage of phenotypic variance explained by individual QTLs ranged from 3.8% to 37.6%. Projection of QTLs on a highly dense gene-based linkage map (6k genes) showed coincidences between QTLs and candidate genes (e.g. encoding terpene synthases) suggesting a regulation at the level of the terpene synthase gene family rather than upstream in the biosynthetic pathway.

Poster number : S2.11

Inheritance of the quantitative traits and bud flushing in Abies alba (Mill.)

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The European silver fir (Abies alba Mill.) is one the most important conifer tree species in Europe. In Romania, silver fir is a main component of mountain forests and has manifold ecological, economical and soil protective functions. Both anthropic and environment pressures on silver fir populations increased in the last decades and therefore focus on quantitative and adaptive traits has become a new priority. Researches in recent decades have revealed a clear change in global climate, which cannot remain without effects on forest ecosystems. However, very little is known concerning the quantitative and adaptive genetic variation of silver fir that will permit species to cope with the climate change.To address these problems, the objectives of this study were: to investigate the genetic variation of growth traits and bud flushing into two progeny tests of silver fir created through controlled pollination and open-pollination, to determine and to compare the genetic parameters from control-pollinated and open-pollinated progenies, to assess the genetic correlations between bud flushing and growth traits, to determine relationship with geographical and climatic parameters of the place of parent origin, and to evaluate the potential for selection.There was a high genetic variation for growth traits and bud flushing in both progeny tests. General combining ability and specific combining ability are very important sources of variation for the studied characters. Dominance variance (σ2SCA) exerted a greater influence as evidenced the σ2SCA / σ2GCA ratios that ranged from: 36 to 19 for root collar diameter, 23 to 14 for total height and 3 to 20 for bud flushing. Narrow-sense individual heritability estimates for full-sib progenies ranged from 0.14 to 0.64, while the full-sib family heritability ranged from 0.09 to 0.40. In the open-pollinated progenies, the values of individual and family heritability were higher ranged from 0.50-0.95 for both. Although the parameters in open-pollinated progeny were greater than the appropriate estimates in full-sib progenies, they do not differ significantly each other at the 0.05 level. Both positive and negative significant effects of general combining ability ($p < 0.001$) were found for growth and bud flushing. High positive genetic correlations were obtained between bud flushing and root collar diameter in full-sib test. Latitude, longitude, altitude, annual mean temperature and annual mean precipitation at the origin places explain together 83% of the bud flushing variation in the full-sib families, while latitude, annual mean temperature and annual mean precipitation explain 97% of the bud flushing variation in the open-pollinated families.Information has an extremely importance in the species breeding program and in the reforestation activity, especially for the transfer of forest reproductive material, in order to maximize adaptability and wood production.

Poster number : S2.12

Exome genotyping and association genetics of environmental adaptation and stress mitigation traits in a clonally tested loblolly pine (*Pinus taeda* L.) population

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Loblolly pine, Pinus taeda L., is the most widely planted and commercially important tree species in the southeastern U.S. To discover new single nucleotide polymorphisms (SNPs) and functional markers available for research and tree breeding, we used genotyping by sequencing (GBS) of targeted exome regions. The exons were captured in a population of 375 trees using the NimbleGen oligonucleotide hybridization probes and then sequenced using the Illumina HiSeq 2500 platform. Oligonucleotide probes were designed for 199,723 exons (≈49 Mbp) partitioned from the loblolly pine reference genome (PineRefSeq v1.01). Bioinformatic analyses demonstrated that the probes covered 90.2% of the target regions. Capture efficiency analyses showed that on average 67.2% of the reads from each tree could be mapped to the target regions, and more than 70% of the captured target bases had at least 10X sequencing depth. A total of 972,720 SNPs were acquired after filtering. Among them, 52.8% were located in coding regions, and 5.3% were located in 5' and 3' untranslated regions. We found that linkage disequilibrium (LD) decays rapidly, with an average correlation coefficient (r2) between pairs of SNPs within single scaffolds decaying to half maximum (r2=0.22) within 55 bp, to r2=0.1 within 192 bp, and to r2=0.05 within 451 bp. The population structure analysis using unlinked SNPs demonstrated two distinct clusters representing western and eastern parts of the loblolly pine range. We will describe association tests that are being conducted to discover markers and genome regions associated with phenotypic traits including height, specific leaf area, carbon isotope discrimination, crown width, nitrogen content, diameter, mean branch angle, as well as with measured earlier pitch canker resistance, gene expression and metabolite traits available from published data.

Poster number : S2.13

Genotyping-by-sequencing targeting the gene space in a natural, wide population of bioenergy *Populus nigra* L.

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WATBIO (www.watbio.eu) is a collaborative research project funded by the European Union seventh framework programme. The project aims to aid the development of improved perennial non-food biomass and bioproduct crops for water stressed environments. Populus holds significant potential as a second generation feedstock for the production of renewable bioenergy and biofuels. Understanding the genetic basis of key bioenergy traits such as biomass yield, wood quality and, due to global climate change, water use efficiency and drought tolerance are research priorities in this species. The work described here employed a natural, wide population of the native European tree P. nigra (black poplar) which comprises more than 1000 genotypes drawn from river populations across the species' western European range. The population has been previously genotyped with a 12K Illumina Infinium array (Faivre-Rampant et al., 2016) but we now report the more extensive genotyping-by-sequencing (GBS) of more than 500 accessions. Previous low-pass sequencing of 51 accessions was available to permit SNP calling and a panel of ~90,000 target single nucleotide polymorphisms (SNPs) was developed according to criteria which identified markers within the gene space. Specifically, 1-4 target SNPs were selected in every annotated gene with priority given to SNPs within exonic regions and the 5' UTR where regulatory regions impacting expression may reside. Probes were designed according to the Nugen (California, USA) protocol such that their 3' end, being an extension initiator, was close enough to the target SNP to ensure read-through capability of the reverse read in a 2x130bp sequencing configuration. Sequencing proceeded in 48-plex using an Illumina Hi-Seq 2500. This

approach was successful in reliably genotyping the targeted SNP from the reverse read but also permitted significant de novo SNP discovery from the forward read providing additional, untargeted markers. In total this provided more than an order of magnitude increase in the number of genotyped SNPs available for this population; from ~10,000 to in excess of 100,000. This novel methodology showed promising features which are applicable to other contexts where cost-efficiency and scalability need to be coupled with knowledge-driven marker selection.The population has been cultivated under short rotation coppice (SRC) at a site in Savigliano, Italy and subject to extensive phenotypic analysis for many of the traits outlined above. These phenotypic and genotypic data are a powerful resource for genome-wide association studies (GWAS) to identify candidate genes and assist the molecular breeding and sustainable intensification of this important bioenergy crop.

Poster number : S2.14

Evaluation of the family-specific growth response of Calophyllum inophyllum to soil moisture

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One of the big problems in understanding the adaptation potential of long-lived trees to future climate change is the difficulty of quantifying genotype by environment interaction. Careful statistical modeling is required to identify the tree growth trend along age, and to evaluate the growth response of genotypes to changing environments such as temperature and soil moisture. To address this issue, we extended the empirical Gompertz growth model, in which tree growth is modelled primarily as a function of age, by estimating the parameters K (the asymptotic size at older ages) and r (the initial growth at younger age). We further assessed the response of families, in terms of the initial growth rate at younger ages, to environmental covariates (soil water content), and estimated parameters of the optimal soil-water level for each family by using a hierarchical Bayesian estimation procedure. To demonstrate our model, we applied it to height growth data of open-pollinated progenies from maternal families of Calophyllum inophyllum planted in the test sites varying soil water contents. We found that there was a considerable variation in the parameter estimates of optimal soil water among families. This suggests that the present approach allowed us to estimate flexibly the growth performance of families along ages by using field monitoring data of test sites, and to obtain useful information about the response of genotypes to soil moisture. Its flexibility will enable more robust testing the growth responses of genotypes to long-term environmental changes.

Poster number : S2.15

Detecting signs of natural selection throughout environmental gradients in holm oak (Quercus rotundifolia Lam.)

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Populations of holm oak (Quercus rotundifolia), are usually part of a multifunctional agro-silvicultural system: the ‘montado’, encompassing among other assets, the provision of energy (coal and firewood) and food (acorns) for domesticated animals. This drought resistant oak is distributed across the Iberian Peninsula and the Maghreb, covering a wide range of climatic conditions, particularly those covering the semi-arid region. Despite this, the area covered by this tree has been decreasing in the last two decades, due to pathogens and deforestation. Although its importance, there is a lack of information concerning the genetic diversity and population structure of the holm oak, which has been known to hybridize with Q. suber, and the whole complex (with Q. coccifera) could act as a diversity

repository within the genus.The goal of this study is to assess the genetic diversity patterns, population structure and genetic variation potentially adapted to environmental factors, in populations of holm oak. We are currently testing several candidate genes, involving in key biotic and abiotic responses, for signatures of natural selection and environmental association. Neutrality tests are used to detect signatures of selection, while environmental association analysis is carried out to test for association between genetic makers with environmental variables. We expect, by the end of this project, to provide information about the genetic signs of natural selection of holm oak in order to contribute for a sustainable management strategies and the conservation evolutionary potential under the current threats such as of climate change.

Poster number : S2.16

Environmental correlates of SNP variation across Quercus robur populations from the species' southern range margin

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Increasing drought stress driven by ongoing climate change has been identified as a major threat for the performance and persistence of Pedunculate oak (Quercus robur) forests in the southern and southeastern parts of the species' distribution range. The potential of oak populations to cope with a rapidly changing environment has important ecological and management implications. It is assumed to depend largely on their standing genetic variation. We investigated variation at 160 SNPs located in candidate genes broadly related to abiotic stresses and bud phenology in Quercus robur populations residing near the species' southern range margin in Croatia, to explore possible effects of local adaptation and their intrinsic capacity to cope with predicted climatic trends at the molecular level. We used complementary methods in order to test for possible natural selection effects: model-based FST outlier approaches and a landscape genomic approach testing environmental associations of SNP-allele frequencies. FST-based tests revealed 8 outlier candidate SNPs showing a divergence on average 6-7 times stronger than the rest of the markers, while 37 SNPs (of which 6 outliers) showed significant environmental associations. Among them, 34 SNPs (including 3 outliers) were significantly related to one or several environmental predictors using a latent factor mixed model, while BayeScEnv detected 3 SNPs among the previous outliers that were associated with two or more environmental variables. According to identified associations, important environmental drivers of potentially adaptive population divergence are suggested to be precipitation seasonality, degree-days below 0 °C and Hargreaves reference evaporation (Eref). The overall genetic differentiation among populations being very low (overall FST ~ 0.013) and the genetic clustering being weak or not significant (depending on the method used), we discuss the biological significance of markers linked to environmental variables representative of regional-scale gradients of precipitation, temperature and aridity. Possible benefits of developing genetically informed guidelines for a climate-change integrated conservation and management of these marginal Pedunculate oak forests are also outlined.

Poster number : S2.17

High density genetic map in oak reveals segregation distortion in oak and allows comparative mapping between oak and chestnut

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A 8k-SNP gene-based infinium array was designed for two European white oaks species Quercus robur and Q. petraea (Lepoittevin et al. 2015) and used to genotype 1,050 offspring belonging to two intra-specific (Q. robur and Q. petraea) and two inter-specific full-sib families. Merging the 8

component genetic linkage maps resulted in a composite gene-based map of 5,460 SNPs (Bodénès et al., 2016). This unique dataset was used to study the extent and distribution of segregation distortion regions among the different parental maps. Main hypotheses concerning the underlying genetic mechanisms will be presented and discussed.Besides, this high density genetic linkage map also constitutes a starting point to study the colinearity with other members of the Fagaceae (e.g. Castanea). Large scale genomic resources are available for Quercus and Castanea, including high density genetic maps (Kubisiak et al., 2012). QTLs detection was performed for several adaptive traits (height growth, bud burst, water-use efficiency, disease resistance) (Casasoli et al., 2006, Brendel et al., 2008, Derory et al., 2009). Comparative mapping clearly indicates a high level of macro-colinearity between both genera. Some QTLs were found to co-localized. We will present and discuss these findings.

Poster number : S2.18

Development of Near-infrared (NIR) spectroscopy calibrations for the genetic analysis of wood properties in natural populations of Populus nigra

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High-throughput analytical techniques to characterize cell wall composition in large-scale lignocellulosic biomass samples from a diverse set of clones / genotypes are essential for unravelling the underlying genetic architecture of wood properties, such as lignin, cellulose and hemicellulose. This, in turn, accelerates the genetic improvement of cell wall composition to reduce biomass recalcitrance during biofuel production. For these purposes, there is an increasing interest in combining Near-infrared (NIR) spectroscopy and multivariate statistical analysis. In this study, we investigated the potential of using this method to predict wood properties, with the aim of applying the predictions to understand the genetic basis of these complex traits in black poplar (Populus nigra L.). Populations representing the natural range of the species in Western Europe were grown in two clonal trials at two contrasting sites under a short rotation coppice (SRC) system and NIR spectra were collected from ca 6,000 wood samples. A subset of 120 calibration samples covering the entire range of spectral variation in the whole population were selected and analyzed using standard methods (wet chemistry, high-performance liquid chromatography, analytical pyrolysis) for wood properties. The absorption spectra and reference values of calibration samples were then employed to develop calibration models at a global scale using partial least squares (PLS) regression and cross-validation.Global models for predicting extractives, C5 / C6 ratio, S / G ratio, xylose / glucose ratio, Py-lignin and soluble lignin had pretty high coefficients of determination (R2cv : 0.71 – 0.86), while the model quality for Klason lignin and common wood sugars (glucose, xylose) was moderate (R2cv : 0.50 – 0.68) and strongly varied depending on the site or coppice rotation considered. The correlations between wood chemical traits and the potential application of NIR analysis for predicting glucose yield following saccharification have also been studied. Our results show the promise of using NIR spectroscopy calibration models for high-throughput phenotyping of wood properties in natural populations of black poplar. Genetic analysis with wet chemistry and analytical pyrolysis data in the 120 calibration samples revealed that the studied wood properties were under moderate genetic control (broad sense heritability ranging from 0.24 to 0.66). The extension of such analyses to the NIRS predicted values in the entire population and their application to association mapping are also discussed.

Poster number : S2.19

Local effects drive heterozygosity–fitness correlations in an outcrossing long-lived tree

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Heterozygosity–fitness correlations (HFCs) have been used to understand the complex interactions between inbreeding, genetic diversity and evolution. Although frequently reported for decades, evidence for HFCs was often based on underpowered studies or inappropriate methods, and hence their underlying mechanisms are still under debate. Here, we used 6,100 genome-wide single nucleotide polymorphisms (SNPs) to test for general and local effect HFCs in maritime pine (Pinus pinaster Ait.), an iconic Mediterranean forest tree. Survival was used as a fitness proxy, and HFCs were assessed at a four-site common garden under contrasting environmental conditions (total of 16,288 trees). We found no significant correlations between genome-wide heterozygosity and fitness at any location, despite variation in inbreeding explaining a substantial proportion of the total variance for survival. However, four SNPs (including two non-synonymous mutations) were involved in significant associations with survival, in particular in the common gardens with higher environmental stress, as shown by a novel heterozygosity–fitness association test at the species-wide level. Fitness effects of SNPs involved in significant HFCs were stable across maritime pine gene pools naturally growing in distinct environments. These results led us to dismiss the general effect hypothesis and suggested a significant role of heterozygosity in specific candidate genes for increasing fitness in maritime pine. Our study highlights the importance of considering the species evolutionary and demographic history and different spatial scales and testing environments when assessing and interpreting HFCs.

Poster number : S2.20

Differential DNA methylation patterns in Quercus suber are related to cork quality

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Cork oak (Quercus suber L) is one of the most important forest species in the Mediterranean basin due to the exploitation of its thick and unique periderm, the cork. When the first or virgin cork is removed, the exposed meristem (phellogen) dies and a new traumatic phellogen is formed in the underlying non-conducting phloem. Cork extractions continue at 9-years intervals allowing the regeneration of new cork layers, but only the third and further developed corks - amadia cork - have industrial uses. Amadia corks show highly variable quality, with lenticular channels and inclusions of lignified phloem ('nails') being the main causes of cork quality depreciation.Epigenetic factors such as DNA methylation have been suggested as a potential cause of the increased phenotypic variation in populations of long-lived species (reviewed in [1]). Recent evidences support the influence of DNA methylation on Q. suber cork quality [2]. In this study we analysed the variability of DNA methylation patterns in virgin and amadia cork and leaf tissues of Q. suber adult trees. Three stands with different edaphoclimatic conditions were selected, and the methylation-sensitive amplified polymorphisms

(MSAP) detected were associated with the most relevant quality traits (cork growth, porosity and 'nails') assessed by image analysis.A total of 339 polymorphic epiloci were found for cork tissues and 303 for leaves. Higher cytosine methylation diversity was found in cork than in leaf tissues suggesting a contribution of the distinct cell differentiation stages undergone by them. When comparing populations we found little epigenetic differentiation for both tissues highlighting a weak role of epigenetics in the adaptation to local environment. When comparing trees producing 'virgin' and amadia corks we found epigenetic differentiation for cork tissues but not for leaves, suggesting some level of epigenetic reprogramming in the traumatic phellogen, eventually responsible for those differences. A similar variation in cork quality traits was found within the studied populations. These traits showed significant associations with at least one MSAP marker, involving 5 loci (2.5% of total) and 4 quality traits (50% of the total), and supporting a role of cytosine methylation in cork quality modulation. Moreover, we found MSAP markers associated with the percentage of 'nails' and porosity, probably indicating a role of cytosine methylation in the regulation of phellogen activity, either involved in localized cell death or in pore production. Our results provide new hints on the role of DNA methylation on the quality of Quercus suber cork.

Poster number : S2.21

No evidence for genetic differentiation between French and Belgian populations of the exotic tree Robinia pseudoacacia

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Robinia pseudoacacia was introduced in Aquitaine for timber production during the eighteen century whereas introduction was more recent in Belgium and probably occurred during the nineteen century. To test for local adaptation of European populations of the invasive tree, a controlled experiment was set up using 2000 seeds sampled in 10 French Aquitaine populations and 10 Belgium populations, cultivated in two climatic chambers set at 18°C and 22°C, conditions corresponding to the average maximum May temperatures in Wallonia and Aquitaine. Phenotypic plasticity and genetic differentiation were assessed through combined plant traits and molecular marker analyses: germination and phenology of young seedlings were monitored, as growth and photosynthetic traits. One individual per family was genotyped using SNP markers. Populations demonstrated a strong plasticity to temperature for all measured traits, the warmer environment being generally more suitable whatever the population origin. No genetic differentiation was evidenced using phenotypic traits and QST indexes; but the QST - FST comparison underlined a slight genetic differentiation at the molecular level. Overall, the genetic structure of these introduced populations demonstrated a high level of admixture with presence of some outlying populations. Still, no evidence for local adaptation was found between Belgium and Aquitaine populations. The genetic structure and diversity of both native and invasive populations of R. pseudoacacia will be further investigated to assess the relative role of admixture, evolutionary processes and tree breeding in R. pseudoacacia introduction and invasion history.

Poster number : S2.22

SNPs vs SSRs: different tales of two markers

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The recent expansion of NGS technologies has allowed the genotyping of large number of SNPs for a multitude of samples at a moderate cost, providing new insights into the evolutionary history, population structure and adaptation of non-model organisms. Moreover it allows a comparison with more traditional markers, the SSRs.Cork Oak populations were sampled and genotyped across 27 locations of the species' range and 21 environmental variables were used for Environmental

Association Analysis. These samples were genotyped for 13 microsatellite loci, and for approximately 45000 SNPs resorting to a combination of multiple genomic techniques (454-RNAseq/MassArray and GBS).Contrary to our expectations the SNPs datasets suggest a West-East structure, while the SSRs data shows an unexpected population structure pattern. Moreover the pattern detected by SSRs only partially corresponds to the pattern obtained from the SNPs dataset. Clusters revealed by SSRs data seem to have higher resolution.The results from traditional molecular markers and NGS based genome scans show the complexity of the evolutionary process and the interplay between structure, adaptation, gene flow and the marker's properties.These results reinforce previous work (Costa, et al. 2011; Paulo, Costa, et al. 2012; Paulo, Pina-Martins, et al. 2012; Modesto, et al. 2014; Pereira-Leal, et al. 2014; Sebastiana, et al. 2014; Pina-Martins, et al. 2016) emphasising the role of selection and gene flow in addition to history and drift as the main driving forces in shaping the pattern of genetic diversity and local adaptation in this species.

Poster number : S2.23

Transcriptomic dynamics during tension wood differentiation in Eucalyptus globulus

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Wood complex anatomical, chemical and physical properties are determined upon composite ontogenetic processes, requiring the spatio-temporal transcriptional and post-transcriptional regulation of a large number of genes. Despite the recent progresses made in understanding the transcriptional regulation of wood formation, our knowledge of the role of miRNA-mediated post-transcriptional regulation of these processes is still very limited. In plants, the post-transcriptional regulation by microRNAs (miRNAs) has been recognized to play crucial roles in diverse biological processes including vascular cambium differentiation. MiRNAs are small (20–24 nt) non-coding RNAs that control a vast array of developmental and stress-related biological processes by down-regulating miRNA translation either by mRNA cleavage or by translational repression.In order to provide new insights on the molecular regulation of cell wall variability we assessed the dynamic of transcriptome (coding and non-coding) during the induction of tension wood in Eucalyptus globulus.Coding transcriptome dynamics during the induction of tension wood of grown- field E. globulus trees were accessed by New Generation Sequencing technology, and annotated taking profit of the recent publicly available genome sequence of E. grandis. Tension and opposite differentiating xylems were collected from three E. globulus clones bent for 1 week to 3/4 weeks. Sequencing was performed using Illumina Hi-Seq 2000 GA. The PE-reads (100bp) aligned against the E. grandis genome sequence, using Top-hat and differentially expressed genes identified by Cufflinks. Among the 93 genes differentially expressed more than a half (53 genes) were shown to be differentially expressed ($P<0.05$) between tension. Non-coding transcriptome was also assessed by NGS using the same samples. The UEA small RNA Workbench (http://srna-workbench.cmp.uea.ac.uk/) were used to identify 164 miRNAS. In addition, degradome libraries of tension and opposite wood collected after one week and 3 and 4 weeks after bending were generated and anaysed, allowing the mass identification of the interactions between miRNAs and theirs target transcript.A pipeline was developed to combine these data, allowing to reveal a reallocation of carbon to active tension wood forming tissues, while it was also put in evidence the role of miRNAs in post-transcriptional regulation of key transcription factors involved in meristematic activity in opposite wood forming tissues. These results and ongoing work will be presented and discussed.

Poster number : S2.24

Genetic architecture of water use efficiency and related traits in maritime pine

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Deciphering the genetic architecture of water use efficiency (WUE) and related traits, such as stomatal conductance and photosynthesis, is especially relevant in view of its implication in plant survival, growth and biomass production under water limiting conditions. Considerable efforts have been deployed on dissecting the genetic architecture of these complex functional traits in maritime pine through the analysis of Quantitative Trait Loci (QTL). Studies conducted on full-sib pedigrees obtained from controlled crosses of different ecotypes from France and Spain (Brendel et al. 2002; de Miguel et al. 2014; Marguerit et al. 2014), have made it possible to identify QTLs for WUE, estimated from gas exchange measurements and carbon stable isotopes analysis as well as for other traits related to photosynthesis.A QTL study was recently performed in a 9 year-old full-sib family obtained from a controlled cross between an individual from Landes (France) and Morocco, which allowed to take into account, compared to already published studies, alleles from a new parent individual, from dry climatic conditions. In spring and summer of 2015, chlorophyll fluorescence measurements were performed on 108 F1 offspring growing in an experimental plot located in Aquitaine (France). A QTL analysis for photosynthesis related traits under different water availability conditions was performed for this family.The projection of previously and newly detected QTLs on the unified gene-based linkage map for the species (de Miguel et al. 2015) allowed to study the co-localization between QTLs. Linkage groups #5, #6, #8 and #12 presented stable QTLs for WUE, while they were identified in independent studies with different genetic backgrounds (French and Spanish ecotypes), under diverse environmental conditions (different water availability) and at different ontogenic stages (from 2 to 15 year-old individuals). Co-localization of QTLs for photosynthesis related traits, under high and low water availability, were also identified between different pedigrees.The use of a high density linkage map as a common framework to gather QTLs from different studies showed a valuable opportunity to check the stability of identified QTLs and discover positional candidate genes potentially involved in the variability of WUE and related traits.

Poster number : S2.25

Exploring genotype-phenotype associations for growth and disease resistance in full-sib families of loblolly pine (Pinus taeda, L.)

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A population of 900 clonally replicated loblolly pine varieties from 35 full-sib families were genotyped using a set of more than 6000 SNPs. Phenotypes included growth, stem form and disease resistance at 6 years of age. SNP markers were used for the estimation of genomic relationships and significant SNP associations were identified for height growth, volume, rust resistance and stem straightness. Resistance to fusiform rust infection caused by Cronartium quercuum (Berk) Miyabe ex Shirai f.sp. fusiforme was heritable (H2=0.44) and highly significant SNP associations were identified using a Bayes CPi analysis. The five (5) most significant SNP loci accounted for 45% of the total variance for fusiform rust infection. Incorporating SNPs into the selection process appears to provide substantial reductions in rust incidence for specific full-sib families. Families with the most significant SNP for rust resistance were inoculated in greenhouse conditions with inocula from 3 regions to assess the stability of resistance across different regions of the Atlantic and Lower Gulf coastal plains. Results from the discovery population, validation trial and potential applications will be discussed.

Poster number : S2.26

Dendroclimatology in a provenance trial: an approach to validate genetic-environment associations in Pinus strobus?

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Under rapid global warming, it is critical for us to better understand the capacity of forest trees to adapt to a changing climate across their lifespan, from seedling to mature stages. However, identifying potential genes involved in genetic adaptation remains a challenge. In this study, we used several methods to detect genes important for local adaptation in Pinus strobus, and propose a method to validate their potential role in growth responses to climate using dendrochronological analysis of trees in a common garden. For this, 153 SNPs from 103 genes, including 44 candidate genes for growth and phenology, were genotyped in 133 populations across the range of P. strobus. Isolation by distance (IBD) and isolation by colonization (IBC) were found to be significant drivers of population structure. Two distinct southern and northern genetic groups were identified that likely originated from different glacial lineages. Isolation by environment did not significantly explain population structure when controlling for IBD and IBC. However, genetic-environment association (GEA) methods and FST outlier tests detected 33 (21.6%) outlier SNPs, suggesting that local adaptation took place in the presence of high gene flow. We combined results across GEA and FST outlier methods and identified six highly supported candidate genes for local adaptation. Then, the growth-climate relationships were determined on a subset of 236 mature P. strobus from 38 provenances representative of the species range in a common garden trial. Many of the highly supported SNPs by GEA and FST outlier tests also covaried with tree growth response to summer drought and summer temperature. We argue that combining various methods to detect selection and validating selected loci using dendroclimatology in field trials could be powerful tools to uncover loci important for local adaptation to climate.

Poster number : S2.27

Signatures of local adaptation at a small spatial scale from the joint analysis of SNP genotypes, environmental data and phenotypic traits in Abies alba Mill.

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Landscape genomic studies provide accumulating evidence for signatures of selection at different spatial scales in conifer trees. These studies aim at detecting loci under selection by jointly analyzing genetic and environmental data. However, the level of commonality among studies are very low. The joint analysis of phenotypic data with genotypic and environmental data might provide a broader picture into adaptation processes and the underlying genetic and phenotypic variation within populations. Here, the main challenge is obtaining phenotypic and environmental data for each single tree. In our study we focused on a single Abies alba population in Bavaria, Germany, to detect signatures of selection by jointly analyzing genotypic, environmental and phenotypic data. We sampled 200 adult trees at two sites that differ in climatic conditions. For all trees, we genotyped 232 SNPs in candidate genes. To characterize the microsite of trees, we determined solar radiation for each tree based on a high resolution digital elevation model (DGM). For the phenotypic characterization, we focused on spring phenology and recorded the date of bud burst for individual trees in 2014 and 2015. We tested for environmental association with latent factor mixed models (LFMM) and for phenotype-genotype associations with MLMs as implemented in Tassel. As we could exclude population substructure as a confounding factor, we could apply the machine learning algorithm random forest which permits to take into account the effects of multiple SNPs and their interactions. With LFMM and random forest, we jointly detected SNPs in three genes that were associated with solar radiation. Two of these genes encode NADH dehydrogenase proteins which are relevant for photosynthesis at low light. As the mountainous topography leads to strong microsite differences in global radiation this could exert a selective pressure on genes involved in

photosynthesis. The temperature sum that accumulated until bud burst differed markedly between sampling sites and showed a high variation within sampling sites. However, this variation was neither related to solar radiation nor to SNPs in candidate genes. Our results indicate that climatic factors trigger local adaptation at very small spatial scales. While the association of phenotype and genotype in natural population remains a challenging task, our results encourage to enhance the efforts to obtain high resolution environmental data from on-site measurements as well as from models based on DGMs or remote sensing.

Poster number : S2.28

Local adaptation in Scots pine (Pinus sylvestris) survival and growth in relation to timing of bud set

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Local adaptation is manifested through better fitness of local than non-local individuals when populations are grown at multiple test sites. Here, young trees originating from 15 Scots pine populations from southern to northern Europe were followed for a period of ten years in two field sites in Finland. The population samples were first studied in a common greenhouse experiment. Timing of bud set in the first year seedlings was recorded, and genotypes in a set of candidate and reference markers were determined for 271 half-sib families. After the greenhouse experiment the seedlings were planted in the two field sites (latitudes 64° 51' and 60° 41'), and survival and growth were recorded. We expected highly differential survival and height growth between populations. We also examined how survival and growth are related to timing of bud set in the first year within populations. Further, we search for genetic associations between the measured traits and the set of SNP markers enriched for candidate genes for latitudinal adaptation.

Poster number : S2.29

Exome Genotyping and Association Mapping in Norway Spruce

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The project aims at providing fundamental information towards the building of an efficient and highly informative breeders toolkit. Currently the Norway spruce (Picea abies) genome has been sequenced and has several scaffolds. To increase the number of known single nucleotide polymorphisms (SNPs) and functional markers available for research and tree breeding, we used the exome capture method for targeted exome regions. The exons were captured in 526 trees using in house designed probes and then sequenced using the Illumina HiSeq 2500 platform. 80 000 hybridization probes were designed from the Norway spruce genome 1.0, with only 40 000 probes being used for analysis. Bioinformatic analysis of the sequence data was performed using the current total Norway spruce genome as the reference genome. A total of 545 546 high quality SNPs were acquired after data processing. Currently, these SNPs are being used for association mapping studies of wood chemistry traits, which include the functional analysis of these traits, population genetics analysis, as well as the development of genetic linkage maps. Association studies have already yielded SNPs significantly associated with density and growth. Two linkage maps have already been developed and will assist with the assembly of the version 2.0 of the Norway Spruce genome.

SESSION 3

Poster number : S3.1

Molecular events in the response to stem inclination in pine

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The molecular response to stem inclination is an intricate mechanism. Particularly intriguing is the role of transcription factors which modulate gene expression, activating specific metabolic pathway. Molecular tools (subtractive libraries, RNA-seq and proteomics) have provided information regarding differentially expressed genes in the response to inclination, which include those associated wall synthesis, lignin biosynthesis cellulose and transcription factors. MADS box is differentially expressed at early time of inclination. This transcription factor is associated to flowering, and the role to stem inclination is not well understood. Microarray analysis have enabled us to compare gene expression by a differentially expressed at early time of response. Genes of the phenylpropanoid compounds (lignin synthesis) are modulated in their expression. On the other hand, bioinformatics tools able us to predict the structure of proteins involved in cell wall remodeling, as well as the transcription factors under study. Xyloglucan endotransglucosidasa/hydrolase suggest that the enzyme has more affinity with hemicellulose type substrates. Moreover, molecular dynamics assay showed the more stable complex formed. Fondecyt Nº. 1150964

Poster number : S3.2

Relative Susceptibility of Pine Families with the Pine Wood Nematode, Bursaphelenchus xylophilus

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Three-year-old 9 open-pollinated pine families were inoculated with the Pine Wood Nematode, Bursaphelenchus xylophilus, at levels of 3,000, 5,000, and 7,000 nematodes/seedling in greenhouse. There were no distinct patterns in latent period among three densities of B. xylophilus in all families. Open-pollinated progenies of Pinus densiflora showed the longest latent period because none of one-year-old needles were wilted until 14 days after inoculation with 5,000 and 7,000 nematodes. Current needles wer not wilted until 14 days after inoculation in all seedlings. The mortality repidly increase from 35 days to 49 days after inoculation. A 3,000 nematodes/100 µL with sterilized distilled water are enough to screen 3-year-old pine seedlings for resistance to B. xylophilus.

Poster number : S3.3

Analysis of the chestnut root transcriptome upon interaction with the oomycete pathogen Phytophthora cinnamomi

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Sweet chestnut, an important forest species for the economy of Southern Europe (fruit, wood and forest biodiversity), covers an area of 2.53 million hectares, including 76,000 ha devoted to 117,000 tons of fruit production (FAO, 2012). Castanea sativa is declining due to ink disease caused by Phytophthora cinnamomi. To elucidate chestnut defense mechanisms to ink disease, we compared the root transcriptome of the susceptible species C. sativa and the resistant species C. crenata after P.

cinnamomi inoculation (1).Four cDNA libraries were constructed, two of them included root samples from C. sativa, inoculated and non-inoculated and the other two libraries comprised samples from C. crenata at identical conditions. Pyrosequencing allowed the assembly of 14,925 distinct genes for C. sativa and 16,118 distinct genes for C. crenata. GO annotation revealed terms related to stress as “response to stimulus”, “transcription factor activity” or “signaling” for both transcriptomes. Differential gene expression analysis revealed that in C. crenata more genes related to biotic stress response upon pathogen inoculation were upregulated than in C. sativa. Those genes were found to be involved in regulation of plant immune response and stress adaptation and recovery. Noteworthy was the overall downregulation of genes in susceptible C. sativa, which may facilitate the pathogenicity of P. cinnamomi.The four sequenced libraries allowed the selection of candidate genes for host resistance to P. cinnamomi and a functional analysis approach is in course for further validation and insights. Also SSR markers were developed from the sequences of candidate genes (2) in order to improve the mapping approach for identification of QTLs related to pathogen resistance in Japanese and European chestnut. The publicly available transcriptome data at Fagaceae Genomics Web (3) and at NCBI Short Read Archive (PRJNA215368) is a valuable contribution to the available Castanea genomic resources.

Poster number : S3.4

Genetic and biochemical responses in Abies nordmanniana needles to attack by silver fir wolly adelgids

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Nordmann firs (Abies nordmanniana) are commonly planted as Christmas trees Northern Europe. They originate from the Black Sea where they naturally co-exist with silver fir woolly adelgids (Dreyfusia nordmannianae). Visible damages caused by the sap sucking adelgids, such as needle curling and needle shedding, can be an economic disaster for Christmas tree growers. The identification and specific cultivation of more tolerant provenances or specific genotypes would reduce the amounts of insecticides currently employed in plantations. An earlier study revealed that different A. nordmanniana genotypes show distinct levels of susceptibility and that tolerance to adelgid feeding is a heritable trait (Nielsen et al. 2002). Furthermore, A. bornmuelleriana, a species (sometimes also considered as sub-species, A. nordmanniana ssp. bornmuelleriana) less frequently planted as Christmas tree appeared to be more resistant to silver fir woolly adelgids. However, detailed knowledge about the genetic architecture of this trait or metabolites involved in insect resistance is lacking so far.In the present study we aimed at identifying candidate genes and plant metabolites involved in the tolerance to adelgid feeding. In 2014 trees with known phenotypes were replicated by grafting them on top of two year old A. nordmanniana trees. In spring 2015 all trees were moved to the greenhouse where part of them was exposed to silver fir wolly adelgids. We compared the response of A. nordmanniana clones with distinct levels of susceptibility, as well as A. nordmanniana and A. bornmuelleriana clones. We characterized levels of gene expression through high-throughput RNA sequencing and analyzed the abundance of volatile and non-volatile compounds in clones with contrasting phenotypes (i.e., showing different degrees of tolerance). Preliminary results revealed several genes that were differentially expressed which might be involved in resistance to the insects.

Poster number : S3.5

Biotic interactions and endogenous rhythmic growth as drivers of oak gene expression

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Biotic interactions and endogenous rhythmic growth with alternating root and shoot flushes constitute major determining factors of performance of oaks in the environment. Quercus robur clone DF159, the

clonal tree model of the platform TrophinOak [1], is used to investigate how a plant coordinates development and biotrophic interactions synthesized under controlled lab conditions.From the oak associated organisms surveyed in the TrophinOak research program, interaction with the mycorrhiza associated bacterium Streptomyces AcH 505 stimulated the formation of mycorrhizal symbiosis and reduced damage by pathogens [2, 3]. The bacterium protected pedunculate oak from pathogenesis by oak powdery mildew Erysiphe alphitoides, and RNA-Seq analysis of bacterium inoculated oaks indicated that priming by AcH 505 does not follow the mechanisms described for nonpathogenic Pseudomonas strains. Namely, the systemic response to the bacterium included the induction of a surprisingly large number of defense-related genes, including genes associated with jasmonic acid/ethylene-dependent and salicylic acid-dependent defense pathways [4].The response to AcH 505 was specifically induced at shoot flush, which indicates that the physiology in roots is rather devoted to growth at root flush while the processes directed towards interactions are in most part attenuated. In contrast to the situation in powdery mildew infection, the gene expression response to AcH 505 was attenuated when a mycorrhizal fungus, Piloderma croceum, was co-inoculated [5]. This indicates that the presence of multiple organisms may either buffer or amplify gene expression responses in oak. Only few genes were differentially expressed by both AcH 505 and co-inoculation, which suggests that the oak coordinates its gene expression responses to AcH 505 in the presence and absence of the EMF by induction of a few, specific microbe associated pattern receptors and transcription factors, i.e. candidate “core genes” of AcH 505 response.When the responses to mutualistic and pathogenic microorganisms were compared, a core group of 43 oak-microbe interaction indicator genes were identified, with genes related to innate immune response, transcriptional control, cell wall dynamics, RedOx processes and secondary metabolism among them. These genes can be used to investigate long-term dynamics of oak DF159 interacting with assemblages of organisms and confronted to a variability in abiotic factors.

Poster number : S3.6

Physiological and morphological response under salt stress of casuarina equisetifolia seedlings

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Casuarina equisetifolia is a fast-growing and nitrogen-fixing tree species, which is used in multiple purpose plantations in South China. The 10 provenances of seedlings were used to test salt resistance. By pot-simulation-stress method (Marcar et al. 2002; Marcar and Crawford, 2011), the physiological and morphological responses of seedlings, including anatomic structure transformation, were investigated under several experiments with different saltine concentrations. Key results as followings:1) The maximum limited concentration of salt resistance for seed germination was 215.6 mmol/L;2) Salt stress dramatically inhibited the seedlings growth. Significant differences were was found both in the provenances and treatments. Realized that response in physiological traits (proline ,MDA, SOD, POD, CAT, K+ and Na+). Ranked salt tolerance of the ten tested provenances.3) The microstructure and ultrastructure transformation under salt treatment were analyzed by SEM and TEM. On the anatomical structure, proved it was a typical salt-secretion halophytic plant. Under salt stress, salt vesicles swelled and ruptured. Atrophy was the important process of adaptation to salt stress, and the mechanism of secreting salt which was done by salt glands was merocrine type. Under salt stress, the chloroplast of branchlets swelled, partly the double membrane fuzzed, class of cavity expanded, stromalamella fracture disordered, and grana lamellae stacking reduced, the chloroplast inner osmiophilic granule size grew as well as quantity. Under high concentration 300 - 400 mmoL/L, near the cell membrane and chloroplast edge the multilayer structure appeared. The multilayer structure was perhaps the special repair mechanism of injury reaction responding to salt stress. Under 400 mmoL/L, which was the highest salt concentration, a small portion of chloroplast broke apart , but a stable whole structure without vacuolization was observed. The vast majority of mitochondria and endoplasmic reticulum and Golgi structure was still intact, cell membrane occurred only presenting slight plasmolysis phenomena.

Poster number : S3.7

Drought-induced acclimation of DNA-hypomethylated poplars

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Plant response to abiotic stress is a main challenge in a context of global climate change. The understanding of physiological as well as genetic/molecular processes controlling plant's response to abiotic stress will help to improve plant breeding. Recently, epigenetic mechanisms such as DNA methylation have been proposed to participate to phenotypic plasticity defines as the different phenotypes for a given genotype in distinct environments is a key process for plant to adapt to their changing environment. This is particularly relevant for perennial plants such as trees that are exposed to repeated fluctuations of their living conditions. In order to assess this question in a perennial plant, we analysed poplar (Populus tremula × Populus alba) RNAi lines inhibited for the DECREASED DNA METHYLATION 1 gene (DDM1, involved in the maintenance of DNA methylation profile). A drought-rewatering experiment was conducted to evaluate the role of DNA methylation in tree phenotypic plasticity. Phenotypic characterization deals with data related to growth, photosynthetic capacities, water-use efficiency, xylem vulnerability to cavitation, xylem anatomy, phytohormones dosage, wood density and Mid Infra-Red spectroscopy (MIRS). Then, epigenomics studies in relation to DNA methylation have been realized: global DNA methylation, Whole Genomic Bisulfite Sequencing (WGBS) and Transposable Elements analyses. Bioinformatics analyses are in progress to identify 'Differentially Methylated Regions' in relation to phenotypic plasticity and the role of DNA methylation in memorizing environmental stress.

Poster number : S3.8

Genomic Resources for Stress-Response in North American Hardwoods

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Enhanced genomic and genetic resources are urgently needed to strengthen tree improvement, stand management, and reforestation efforts to address the increasing number of forest health issues and epidemics. The breadth of tree species and environmental stressors involved have posed a major hurdle to resource development, however. The Hardwood Genomics Project's goal therefor was to generate genetic and genomic resources for as broad a taxonomic distribution of hardwood tree species as possible. Over 4 years, the project generated > 245 Gbases of EST sequence data for black walnut (BW, Juglans nigra), blackgum (BG, Nyssa sylvatica), green ash (GA, Fraxinus pennsylvanica), honeylocust (HL, Gleditsia triacanthos), northern red oak (NRO, Quercus rubra), sugar maple (SM, Acer saccharum), yellow-poplar (YP, Liriodendron tulipifera), and sweetgum (SG, Liquidambar styraciflua) from tissues of seedlings grown in greenhouses under various abiotic stress conditions (drought, heat, cold, wounding, and ozone), and multiple tissues from their parent trees. Genomic and EST-based SNPs and SSRs were identified for all of the species, and were used for

preliminary genetic diversity estimates, to develop mapping populations by paternity analysis, and to construct genetic linkage maps (for NRO, BW, GA, YP, and HL). BAC libraries were constructed for NRO and BW, from which 192 NRO and BW BAC clones harboring candidate genes associated with dormancy, biotic stress or flowering were sequenced. Differential gene expression and network analyses focusing on ozone-stress are uncovering both shared and species-specific responses among the species in defense, photosynthesis, mitochondrial respiration, and senescence. All data are publicly available and searchable online at the project website (www.hardwoodgenomics.org) and NCBI. This project was supported by a grant from the National Science Foundation's Plant Genome Research Program (IOS-1025974).

Poster number : S3.9

Characterizing molecular regulators at the origin of the protective periderm barrier formation

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The phellogen is responsible for the formation of the periderm that replaces the epidermis in the plant stem and roots during secondary growth. Phellogen initials give rise to cells that differentiate into phelloderm towards the inside of the stem and into phellem (cork) towards the outside [1]. Phellem cell walls are highly suberized which represents a physiologically important strategy that confers protection from abiotic stresses such as drought, and from the invasion of pathogens. The molecular mechanisms underlying phellogen functioning and periderm formation are largely unknown.We show that a Populus SHORT-ROOT-like gene is expressed in the phellogen and is involved in secondary growth. Three SHR-like genes have been identified in Populus, PtSHR1, PtSHR2A, and PtSHR2B [2] where PtSHR1 is considered to be the putative ortholog of the AtSHR. Unlike PtSHR1, that is expressed throughout the cambial zone of Populus, PtSHR2Bprom:uidA expression was detected in the phellogen. Additionally, PtSHR1 and PtSHR2B expression patterns markedly differ in the shoot apex and roots of in vitro plants [3]. Transgenic hybrid aspen expressing PtSHR2B under the 35S constitutive promoter showed overall reduced tree growth while the proportion of bark increased relative to the wood. RT-qPCR revealed increased transcript levels of cytokinin metabolism and response-related genes in the transgenic plants consistent with an increase of total cytokinin levels. This was confirmed by cytokinin quantification by LC-MS/MS [3]. Our results indicate that PtSHR2B appears to function in the phellogen and therefore in the regulation of phellem and periderm formation, possibly acting through modulation of cytokinin homeostasis. Furthermore, this work points to a functional diversification of SHR after the divergence of the Populus and Arabidopsis lineages. This is supported by the identification and partial characterization of similar SHR-like genes in the tissues of cork oak, a species well known for its unusual ability for sustained phellem (cork) production as a result of the activity of the phellogen during the whole lifespan of the tree. These findings may contribute to the dissection of this adaptive trait in cork oak, which is simultaneously at the basis of a highly profitable cork industry.Acknowledgements: we thank Paula S Campos (INIAV) for help in plant growth measurements and Eva Hirnerová in phytohormone analyses. FCT is acknowledged by projects PTDC/AGR-GPL/098369/2008, IF/01168/2013, and grants SFRH/BD/44474/2008 (ALM), SFRH/BD/30074/2006 (AM). ON was funded by the Ministry of Education, Youth and Sports (National Program for Sustainability I Nr. LO1204, and 'Návrat' program LK21306).

Poster number : S3.10

Genetic analysis of *Fagus sylvatica* populations across a precipitation gradient in Switzerland using microsatellite markers and SNPs in candidate genes

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European beech (Fagus sylvatica) is one of the most important forest tree species in Europe and could be affected by global climate change. The most likely climate scenarios for Europe predict less precipitation, higher annual mean temperatures and more frequent and prolonged drastic droughts during summer that can badly affect beech survival. In addition, increasing temperatures could promote earlier flushing in spring and later bud set in autumn, potentially increasing the risk of frost damage. Consequently, the genetic adaptation potential of this species to climate change is of great interest. The main objective of this study is to assess the connection between genetic variability and climate adaptation related traits. For this purpose both saplings and adults from beech populations located along two precipitation gradients in Switzerland were sampled. The saplings were subjected to experimentally controlled soil water shortage, and their morphological and physiological responses to water deprivation were evaluated. All individuals were genotyped for 13 microsatellite markers and 70 SNPs in 23 candidate genes associated with drought related traits. Morphological and physiological data suggested that saplings belonging to populations with lower precipitation were less affected by water shortage. Analyses of microsatellite and SNP markers demonstrated that the investigated populations have high genetic diversity and low but significant population differentiation. Interestingly, populations occurring in sites with low precipitation had the highest pairwise differentiation. FST outlier analysis showed that one SNP in the NAC transcription factor gene and one SNP in the Cysteine proteinase gene are likely under positive selection. These results together with association analysis of phenotypic traits and genetic variability (in progress) will help us better understand the genetic variability underlying drought tolerance in Fagus sylvatica, and thus, gain insight into the adaptive potential of this important species.

Poster number : S3.11

Genetic differentiation and Phenotypic plasticity of Maritime pine under water-stress conditions

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Phenotyic plasticity and standing genetic variation is of fundamental importance in evolutionary, population, conservation, and global-change biology. Maritime pine is a conifer species with high differences in plasticity and genetic variation at different levels (populations, families and individuals) and among traits. However, it is still necessary to understand the adaptive significance or the plasticity, and also the tradeoffs among plasticity and other traits. We therefore checked the extent of standing variation and phenotypic plasticity at three different levels (population, families and individuals) in d13C (a WUE related trait), growth, and reproductive output (estimated through the number of female cones). We sampled ca. 200 trees in two contrasting experimental sites, and for two consecutive years. The sampling scheme included 12 populations covering the distribution range of the species, 119 families and 8 trees/family. The results showed a large genetic variation in the isotopic composition (d13C) of the material assayed (h2: 0.31-067 depending of the site and year), with an important GE interaction, and phenotypic plasticity (1 order of magnitude among families for max-min value of the different environments). We explored the adaptive significance of such differences by using reproductive output and growth as proxies to fitness, and by regressing the values with environmental variables of the sites of origin (R2: 0.13-0.40 ,with Mean Annual Temperature and Rainfall).

Poster number : S3.12

Genetic basis of biotic interactions among tree neighbours: identifying the most influential neighbours, and extension of the direct-indirect genetic effects model to multiple traits, ages and sites

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Recent developments in quantitative genetics suggest that interactions among conspecifics may change conceptually the inheritance and response to selection (Bijma 2011). In forest trees, such indirect genetic effects (IGEs) may occur by competitive interactions and exposure to disease infection (Costa e Silva et al. 2013). Population studies of IGEs have been focused on a univariate approach enabling, for a given trait, the estimation of a covariance between direct and indirect additive genetic effects, which is a key determinant of the impact of IGEs on total heritable variation (Bijma 2011; Costa e Silva and Kerr 2013). However, as selection operates on complex phenotypes, it is important to consider genetic correlations among traits to achieve an integrated understanding of the potential for genetic response to selection.We developed a procedure to identify a subset of the eight first-order neighbours that had a major impact on a focal tree, and then extended a univariate direct-indirect genetic effects model to the multivariate level. When compared with the 8-tree full neighbourhood, modelling the average effects of IGEs using a reduced neighbourhood with the most influential neighbouring positions often resulted in increases in the magnitude of the estimated indirect genetic variance, as well as enhanced the overall significance of the IGEs, while narrow-sense heritability estimates remained virtually unchanged. Modelling pairs of traits, or ages or sites within a trait, provided novel insights into the genetic architecture of the studied population. A bivariate model enabled an explanation for why IGEs arising from the increased probability of neighbour infection did not cause reduced growth of neighbours, despite such adverse fitness consequences being evident at the direct genetic level. The strong, genetic-based competitive interactions amongst trees for diameter growth were established early in the stand development, and the heritable competition effects remained positively correlated over time and across sites.

Poster number : S3.13

Dissecting the molecular responses potentially involved in the tolerance of two Ulmus minor genotypes during Ophiostoma novo-ulmi colonization

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Dutch elm disease (DED) is a vascular wilt disease caused by the fungi Ophiostoma novo-ulmi. This pathogen spreads through xylem vessels inducing their blockage and cavitation, resulting in foliar wilting and subsequent tree death. The outbreak of two pandemics during the last century severely affected North American and European elm populations. Currently, many trees are still dying due to the difficulties to control this highly virulent disease. Thanks to the efforts carried out by the Spanish Elm Breeding Programme, the response during O. novo-ulmi colonization of hundreds of Ulmus minor genotypes has been analysed. This allowed us to select well-characterized genotypes with contrasted tolerance to DED as suitable material for dissecting the molecular responses potentially involved in tolerance to disease.Three elm genotypes with remarkable differences in tolerance to DED were selected for this study. M-DV1 (Dehesa de la Villa, Madrid) was selected as it shows high susceptibility to O. novo-ulmi, whereas AB-AM2.4 (Almansa, Albacete) and M-DV2.3 (Dehesa de la Villa, Madrid, registered genotype1) were selected as tolerant and highly tolerant genotypes, respectively. Five

years old plants were inoculated with a highly virulent local strain of O. novo-ulmi (Z-BU1). Two-year-old twigs were collected at a height of 2 m and at 1, 3, 7, 14 and 21 days post inoculation from both control and infected trees. Stems were immediately frozen in liquid nitrogen and stored at -80º C. Samples were hybridized to an oligonucleotide microarray (Agilent 8 x 60K, Agilent Technologies), which was designed using a transcriptome constructed with samples exposed to different abiotic and biotic stress2. A set of 300 genes were exclusively identified in AB-AM2.4, and 167 in M-DV2.3, while 50 genes were identified in both genotypes. Notable increases in level of genes involved in response to biotic stimulus (GO:0009607) were identified, including PR and LRR proteins and transcription factors. These results suggest that U. minor tolerance to O. novo-ulmi is related to the differential expression of these genes.AcknowledgementsThis research was funded by the Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA) and by the Spanish National Research Plan (AGL2012-35580).

Poster number : S3.14

Molecular study of drought response in the Mediterranean conifer Pinus pinaster Ait.

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Pinus pinaster Ait. (Maritime pine) is an important Mediterranean conifer subjected to recurrent drought periods, which according to climate change predictions will increase. Notwithstanding its relatively small geographical range, this species, which is found along a rainfall cline, is characterized by a significant genetic and adaptive diversity.Molecular processes involved in Maritime pine response to drought were analyzed targeting progeny individuals from an ad-hoc designed full-sib cross (Gal1056xOria6) that segregates for this trait.A collection of cDNA libraries for coding and non-coding RNAs (ncRNA) were constructed using as template RNAs extracted from different tissues (roots, stems, needles) from four genotypes (tolerant and sensitive) vegetatively propagated and subjected to drought. Twelve cDNA libraries were sequenced using GS FLX Titanium. A total of 2.416.362 reads from all libraries were cleaned using SeqTrimmNext and mapped separately against the P. pinaster reference ProCoGen transcriptome. Non mapped reads were de novo assembled obtaining 8.700 transcripts that were integrated with the reference transcriptome. A total of 73.247 transcripts were compared with proteins from model plants, including conifers, using BlastX. Transcript annotation was performed using Blast2GO. Differentially expressed genes were identified in each tissue by comparison between control and drought stressed plants using Kal's z-test. Genes showing p-value < 0,005 were selected. Comparison between sensible and tolerant genotypes showed 439, 253 and 135 genes overrepresented only in roots, stems and needles of tolerant genotypes, respectively.Twelve libraries of small ncRNA were also prepared and sequenced by Illumina. A total of 32.702.657 distinct reads were obtained and processed using a dedicated workflow for small ncRNA analysis (https://github.com/forestbiotech-lab/sRNA-workflow). After identification of conserved and putative novel miRNAs, and tasi-RNAs, gene expression analysis revealed that the roots and the needles were the tissues with the highest and lowest number of upregulated sRNAs, respectively, in both the sensitive and the tolerant genotypes. A few candidate miRNAs putatively involved in drought responses were highlighted as a result of this analysis.A set of differentially expressed genes and sRNAs were selected for further qRT-PCR studies.SNPs associated to selected candidate genes were identified in the transcriptome and mapped in the reference genetic map to search for association with previously identified QTL Additionally, modification of cytosine methylation during drought response was studied AcknowledgementsThis work was supported by the Spanish grant PINCOSEQ

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Poster number : S3.15

Acclimation of Populus to wind: kinetic of the transcriptomic responses to single or repeated stem bending

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In Europe, 5 % of the annual timber harvest is lost due to strong winds. Due to global climatic changes, increases of strong wind episode frequency is expected while chronic wind speed during the vegetation period - useful for the acclimation of plant to wind - may decrease [1]. It is important to understand how trees may or may not acclimate to these new wind regimes. The present study aims at deciphering the signaling processes involved in tree responses to mechanical solicitations.Wind induces stem flexions perceived by trees, that trigger a growth acclimation response during several days [2]. Up to date, only a few studies analysed the underlying mechanisms of the tree responses to wind at the transcriptomic level, rather focusing on few genes or at a single time after the mechanical solicitation. In this study, we analysed the kinetic responses of the whole transcriptome of Populus after one controlled bending of the stem. More than 3000 genes were differently regulated in the stem during the three days following the bending. By combining clustering methods with Gene Ontology enrichment, we identified different expression profiles, allowing the characterization of a succession of biological processes, potentially involved in the phenotypical response such as jasmonic acid mediated signalling pathway, cell wall organization or biogenesis, or the inhibition of photosynthesis.In natural conditions, wind bends stems repeatedly and this recurs on time scales varying from seconds to days. By applying repeated stem flexions at a sub-saturation level coupled with kinetics analyses of the responses to each successive bending, we had demonstrated a rapid reduction in responsiveness of poplar growth responses [3][4]. This suggests a stress imprint or an attenuation phenomenon in response to recurrent mechanical solicitations, as it was described in responses to other abiotic stresses. To unravel this phenomenon at molecular level, we compared transcriptomic data obtained after one bending to transcriptomic data obtained after two bendings (24 h interval). We identified three responses patterns among the genes: (i) 96% of the genes immediately regulated after a first bending were less (or not) regulated after a second bending revealing the importance of the attenuation effect; (ii) Some genes were similarly regulated whatever the number of bending; (iii) About 20 genes were newly regulated, suggesting that they were regulated only after two bendings. This fine regulation of the mechanical signalling pathway may allow the plant to not over react to successive bendings.

Poster number : S3.16

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

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Climate change, which is leading to increased mean temperatures and is expected to decrease annual precipitation, may be too rapid to allow for adaptation to stress in long-lived forest trees. Forest trees have various strategies to cope with drought stress and their responses involve complex molecular mechanisms. The great variability in genotype x environment interactions increases the difficulty in performing molecular studies to decipher the genetic basis of drought response in mature forest tree species. Pinus halepensis (Aleppo pine), is widespread in the Mediterranean basin and is one of the most drought-tolerant pine species. Mature trees from a semi-arid area with sub-optimal growth conditions were selected for clonal propagation through cuttings in order to reduce the genetic

variation effect and to maintain mature tree properties. We used a high-throughput experimental system which monitored 48 plants every 3 minutes in parallel and calculated whole-plant transpiration rate, daily biomass gain, water use efficiency, whole-plant stomatal conductance, soil moisture levels, ambient radiance and vapor pressure deficit (VPD). In addition to the physiological measurements, a hormonal profile of selected samples was determined. The experiment included 3 environments: baseline, drought and recovery. Needles were collected for molecular analysis every 3 days. Total RNA extraction and cDNA libraries were prepared from drought-stressed and well-watered clones from 5 time points according to the physiological stage, i.e. baseline, stomatal closure, maximum drought, post-irrigation and recovery. A Drought-stressed P. halepensis transcriptome' was de novo assembled using paired-end RNA-seq and was compared to the published P. halepensis transcriptome. RSEM analysis revealed a total of ~4,000 differentially expressed non-redundant transcripts. GO Enrichment results suggest that drought response involves down-regulation of processes such as cell cycle, cell growth, transcription, response to endogenous stimuli, RNA metabolism and biosynthesis, and up-regulation of localization, chloroplast related and homeostasis processes. A multi-disciplinary approach utilizing topophysis to achieve high-throughput physiological measurements, RNA-seq and comprehensive hormonal profiling offers a better opportunity to identify drought-related genes in forest trees. This is a new tool for improving drought stress tolerance of forest trees in advance of the aridity predicted for coming generations.

Poster number : S3.17

Sensitivity and potential of Terminalia tomentosa Roxb towards different gamma irradiations exposure regimes at early seedling growth phases

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The seeds of Terminalia tomentosa Roxb were collected from Achanakmar Amarkantak Biosphere reserve, Central India. The air dried seeds were exposed to different gamma irradiations doses (10KR, 20KR, 40KR and 60KR) in Continuous (C) and Fractionated (F) pattern using 60 Co source. The effect of different gamma irradiation doses were examined to analyse the sensitivity and potential on germination, growth and vigor parameters of Terminalia tomentosa. Increase in the germination and growth parameters under the lower continuous and fractionated doses of gamma irradiation indicate the potential of the lower doses of gamma irradiation in the early growth improvement of Terminalia tomentosa. In contrast, the higher doses of gamma irradiation have a retardant effect on germination, growth and vigor parmeters thus the tree was showed higher sensitivity towards these dose levels. It was observed that the fractionated dose pattern cause more inhibition in different parameters than the continuous doses.The 10KR continuous dose level showed the best acceptability and maximum enhancement towards all parameters for germination record. The mean values under different treatments were compared using Duncans Multiple Range test at 0.05% level of probability. A statistical significant variation existed and was confirmed by using ANOVA at 0.05% level of significance under the different gamma irradiation regimes.

Poster number : S3.18

Genomic comparison of Geosmithia morbida isolates from genetically distinct clusters

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Geosmithia morbida is plant pathogenic fungus associated with a disease complex of walnuts, Juglans spp., known as thousand cankers disease (TCD). The fungus moves within the phloem, yet is most effectively spread throughout the tree by its vector, Pityophthorus juglandis, walnut twig beetle. Multiple dark brown- to- black cankers coalesce to girdle twigs and branches. Severely infected trees initially display wilting and yellowing foliage, branch dieback and eventually tree mortality within 3–4 years after symptoms develop. TCD was originally described from the western US and now has expanded to the native, eastern range of black walnut in the US. TCD has recently been discovered in

northwestern Italy on both black and native J. regia, English walnut. If TCD becomes established throughout the native range of black walnut, economic, environmental and social consequences could be significant as the current estimated value of standing J. nigra stock in the US alone is $568 billion. Although Geosmithia is distributed worldwide, G. morbida, and most recently, G. pallida, are the first members of the genus to function as plant pathogens. Currently, there is little scientific knowledge regarding mechanisms of host-pathogen interactions, and there are no known host resistance mechanisms to TCD. Our previous research has indicated high genetic diversity among G. morbida subpopulations with evidence of gene flow and a significant correlation between geographic and genetic distance. Bayesian clustering analyses identified five distinct genetic fungal clusters across native and non-native regions of black walnut with no evidence of sexual reproduction or genetic recombination. These findings supported the hypothesis that G. morbida has been disseminated to different regions of the US multiple times from multiple sources. To further our understanding of G. morbida pathogenicity, we used a comparative genomic approach tocharacterize regions within the G. morbida genome that have experienced selection and rapid evolution since diverging from a recent non-pathogenic ancestor. Combined with a recently published reference genome, the re-sequencing and assembly of representative isolates from our previously identified genetic clusters will enhance the utility of the genome for comparative research within the genus and across fungal lineages. Genome-enabled research approaches can corroborate evidence and identify mechanisms of G. morbida pathogenicity and host defenses, thus providing a platform for development of informed and effective disease management strategies.

Poster number : S3.19

Blending Ecology and Evolution using Emerging Technologies to Determine Species Distributions with a Non-native Pathogen in a Changing Climate

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A collaborative team of researchers from the US and Mexico have begun an exciting new research project funded by The National Science Foundation's Macrosystems Biology program. The project is to study ecological and evolutionary processes affecting the distribution of southwestern white pine, an important tree species of mixed conifer forests in the Southwest and Mexico. Southwestern white pine sustainability is threatened by changing climate, and a non-native tree disease, white pine blister rust. White pine blister rust causes extensive tree decline and mortality where it occurs in North America, including where it overlaps with southwestern white pine, an ever-expanding area. Climate may change too rapidly for southwestern white pine to adapt. The dual threats of climate change and invasive species make forecasting future tree distributions across continental scales an urgent challenge. The goal is to determine how gene movement among populations, adaptation to disease and drought, heritable changes beyond DNA mutations, and a changing environment interact to govern the success of southwestern white pine. This project will develop tools to help forecast and manage the future of the species, including genomics, common gardens, tree disease resistance testing, engineering and technology innovation to measure drought tolerance, and computer modeling in landscape ecology and genomics. The research team will use the Southwest Experimental Garden Array, a new genetics-based research platform that allows scientists to quantify the ecological and evolutionary responses of species to changing climate conditions. The research approach will provide a prototype for forecasting complex system behavior applicable to other systems, including those facing similar ecological challenges and will contribute directly to the conservation of southwestern white pine while strengthening cross-border research and management efforts in forest conservation.

Poster number : S3.20

Transcriptomic analysis reveal new strategies to face long exposure to low temperatures in Norway Spruce.

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Cold acclimation in plants is a complex phenomenon that involves a number of different stress-responsive and metabolic pathways. Most gene expression studies have addressed short-term cold acclimation responses in herbaceous plants while few have focused on the long-term cold responses, and even fewer have investigated perennial evergreens such as conifers. With the aim of characterizing the transcriptome involved in cold acclimation in Picea abies (Norway Spruce), we performed RNA-Seq analysis of needles and roots from two years old plants. To characterise the short-term cold acclimation response, plants were shifted from 22℃ to 4℃ for periods of up to 10 days. To assess second phase acclimation, these same plants were then exposed to freezing (-4℃) for periods up to a further 10 days. We identified 8 significant gene clusters responding in roots and 5 in needles. These clusters were populated by co-expression and used to perform a membership analysis, which generated a total of 1377 high confidence cold-regulated (COR) genes in both tissues. Our results indicate that there are coordinated cold acclimation stress gene responses in Spruce and more genes respond to cold in roots than in needles. However, in general terms the fold changes were greater in needles than roots during both cold acclimation phases. In needles we identified a cluster of 130 genes enriched in process related to stress responses and cell wall and lipid metabolism that in the chilling phase respond rapidly and positively to cold treatments after 6 hours at 4℃ but then reduce its expression progressively below the control levels. This cluster subsequently increases expression again upon exposure to freezing, indicating that this early response to cold cluster is also necessary for freezing tolerance. Comparing gene expression responses of needles against Arabidopsis thaliana leaves during the initial cold acclimation phase, our results show that the transcriptomic response of Spruce is much slower than Arabidopsis. Based on a comparative genomic analysis between Picea abies and Arabidopsis using high quality proteomes we identified many Spruce COR genes that were previously involved in cold response in other plant models but also we identified new genes responding to cold which previously were not associated with cold response, providing novel insights into conifer strategies to face long exposure to low temperature.

Poster number : S3.21

Vulnerability to drought-induced cavitation in poplars: synthesis and future opportunities

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Vulnerability to drought-induced cavitation is a key trait of plant water relations. Here, we summarize the available literature on vulnerability to drought-induced cavitation in poplars (Populus spp.), a genus of agronomic, ecological and scientific importance. Vulnerability curves and vulnerability parameters (including the water potential inducing 50% loss in hydraulic conductivity, P50) were collected from 37 studies published between 1991 and 2014, covering a range of 10 species and 12 inter-specific hybrid crosses. Results of our meta-analysis confirm that poplars are among the most vulnerable woody species to drought-induced cavitation (mean P50 = -1.44 and -1.55 MPa across pure species and hybrids, respectively). Yet, significant variation occurs among species (P50 range: 1.43 MPa) and among hybrid crosses (P50 range: 1.12 MPa), within species and hybrid crosses (max. P50 range reported: 0.8 MPa), as well as in response to environmental factors including nitrogen fertilization, irradiance, temperature and drought (max. P50 range reported: 0.75 MPa). Potential implications and gaps in knowledge are discussed in the context of poplar cultivation, species adaptation and climate modifications. We suggest that poplars represent a valuable model for studies on drought-induced cavitation, especially to elucidate the genetic and molecular basis of cavitation resistance.

Poster number : S3.22

Drought-induced plasticity in daily stem radius fluctuations and relationships with xylem hydraulics in hybrid poplars

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Radial stem variations are a great source of tree physiological and ecological information. Time series of stem radius fluctuations using point dendrometers can provide information about radial stem growth and tree water relations with unprecedented quality and high temporal resolution. It is well known that climatic drivers such as the evaporative demand or water availability exert a strong control over the daily fluctuation patterns. The extent of within-species variations and the interaction with the environment remains however poorly documented. Here, we present stem radius fluctuations among eight 3-year-old Populus deltoides × Populus nigra genotypes grown in a common garden under two distinct water regimes. Daily patterns were analysed for one growing season. From daily patterns, we extracted the maximum daily shrinkage (MDS) which is mostly related to the capacitive discharge of outer bark elastic tissues and the day-to-day difference in stem radius over time (dR) which is mostly related to cambial growth. The duration of each daily cycle and individual phase (stem contraction, stem expansion and stem radius increment) were also estimated. Differences among genotypes, genotype × drought interactions, and relationships with other aspects of tree water/carbon relations such as xylem vulnerability to cavitation, growth and water-use efficiency will be presented and discussed in line with potential implications for poplar cultivation.

Poster number : S3.23

Population differences in cork oak for growth and survival under contrasting environmental conditions

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In the face of climate change, understanding the adaptation potential of woody species to cope with different environmental stress events (e.g., drought, frost, pests and diseases) is required to develop sustainable forest management practices. In this context, a key issue when pursuing reforestation actions is to know whether current locally adapted provenances will still show good survival and growth under changing environmental conditions.Cork oak (Quercus suber) is an economic and ecological valuable tree species in the Portuguese forest ecosystems, with a wide distribution across the Mediterranean basin. Since the species' broad natural distribution encompasses contrasting climate and geographic conditions, a high level of provenance variation can be expected in fitness and functional traits through genetic adaptation and/or phenotypic plasticity. In this context, cork oak provenance trials represent a valuable resource to assess the level and pattern of variation between and within provenances, while also allowing the identification of the most adapted seed sources to be used in afforestation activities. Thirty five provenances, covering the entire range of the species' natural distribution, were tested in multi-environment trials established in 1998 under different environmental conditions in Portugal. At age 14 years from planting, height growth, aboveground diameter and survival were assessed in two of the provenance trials that were located at sites with contrasting climate and altitude. Using a multi-site linear mixed model, preliminary results revealed highly significant differences between site means, as well as highly significant provenance variance within sites, for all the analyzed traits. Provenances originating from North Africa, in particular Moroccan provenances, presented the highest survival rates and were the fastest growing in both

trials, and thus performing better than local provenances. Using climate data obtained from the sites of provenance origin, multivariate analyses were applied to classify provenances into “climatic groups”, and then we have explored whether the magnitude and significance of previous estimates of model parameters were affected by including climatic group as a fixed term in the linear mixed model.

Poster number : S3.24

The transcriptional response of Picea abies seedlings to prolonged elevated temperature and carbon dioxide

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Global atmospheric CO2 levels are continuing to increase in parallel with increases in temperature, especially in boreal regions. While there is a growing body of evidence for the effects of, and responses to, elevated CO2 for angiosperm tree species, the effects have been less well considered for coniferous tree species. Such knowledge is essential to inform forestry practice, breeders and modellers of global climate change given that conifers are the dominant species in boreal regions and are therefore experiencing the most rapid and dramatic changes in climate. We aim to develop an understanding of tree-level responses to each abiotic stress as well as understanding whether elevated CO2 can modulate the effects of increased temperature. We grew Picea abies seedlings in climate chambers to study of the combinatorial effects of ambient and elevated CO2 and two elevated temperatures. The carbon dioxide concentrations were 400 ppm (ambient) and 750 ppm (elevated) respectively, and the temperature treatments were ambient temperature (T), ambient temperature plus 4℃ (T+4) or ambient temperature plus 8℃ (T+8). We harvested needle tissue in five-year old P. abies seedlings that had been exposed to the treatment conditions for three seasons and performed replicated RNA-Seq studies, making use of the P. abies genome resource. To complement these transcriptional data we also collected a range of physiological and anatomical data to relate changes in gene expression to those at the phenotypic level. Our findings revealed distinctive and detrimental changes accompanying the shift from T to T+4 and T+8 but there was little evidence to support an impact of elevated CO2 on the transcriptome or to support the notion that CO2 has an effect of ameliorating the impacts of high temperature. Increased temperature influenced gene ontology categories including heat responses, oxidative stress, cell wall physiology, growth and water-use. These findings were supported by evidence from the physiology and anatomy studies. Before extending our studies to consider within- and between-population differences in response to elevated temperature and CO2, and the associated implications for breeding, we require an in-depth understanding of the abiotic stress responses within the species. While this experiment used a collection of seedlings from mixed backgrounds in Picea abies that is representative of current forestry practice, future work is also anticipated to explore genetic differences in response to these treatments.

Poster number : S3.25

A new insight into the response of phloem and xylem to increasing growth temperature and elevated CO2 concentration, in Picea abies

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Young (2 years old, at the beginning of the experiment) Norway spruce (Picea abies) trees were grown for 3 seasons under elevated temperatures and elevated carbon dioxide concentration to determine the impact of altered climate on wood growth and biomass quality. The experiment was set up in a full factorial design consisting of three temperature treatments – ambient, ambient +4℃ and ambient +8℃ – and two CO2 concentrations – 400 ppm and 750 ppm. Chemical and anatomical analysis of the wood and fiber properties did not yield striking treatment effects within the time period of the experiment. However, looking into the response to the treatment conditions the the level of gene expression within the active phloem and xylem tissues, revealed a pronounced activation of a stress response cascades, that was enhance with increasing temperature and was not ameliorated by elevated CO2. Our results indicate that even moderate increases is seasonal growth temperature imposes a prolonged and consistent pressure of environmental stress on these wood forming tissues.

Poster number : S3.26

Impact of potassium and sodium fertilization on secondary cell wall formation in Eucalyptus grandis: integration of transcriptomic and metabolomics data

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Fast growing Eucalyptus tree is well adapted to various soils and climate environment, but its growth varies strongly according to these factors (Merchant et al. 2007). Thanks to potassium fertilization to supply deficient soils (Almeida, 2010), plantation productivity in the south of Brazil is one of the highest in the world. But potassium consumption is rising with negative consequences from an economical point of view, resources are limited, and plantations are facing more and more drought events. The development of more sustainable cultural practices requires an improved understanding of plant response to water stress in interaction with mineral nutrition. In particular, the replacement of potassium by low amounts of sodium has shown to increase above-ground biomass by 56% compared to unfertilized control plants (Almeida, 2010). We aimed to characterize the effect of water availability and nutrition supply on wood formation and quality. An experimental design was set up on field with a highly productive Eucalyptus grandis clone planted in a split-plot design (Epron, 2015; Battie-Laclau, 2016), with 2 factors tested in interaction: the water availability with 100% rainfalls (+H2O) or 63 % of rainfalls (H2O) set up with rainfall exclusion system, and the fertilization with +K, +Na or Control (-K-Na). We analyzed wood structure and composition by histochemical staining and biochemistry (Klason, Thioacidolysis and Analytical Pyrolysis). We performed large scale analysis of transcriptome (RNAseq) and metabolome (GC-MS) in developing xylem. These data were integrated using multivariate statistical analyses and results will be discussed in the present poster.

SESSION 4

Poster number : S4.1

Beech transcriptome data: "I am stressed!"

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The climate change can significantly modify the composition and structure of forests. It is expected that European beech (Fagus sylvatica L.) could be sensitive to climate changes. Regional climate models predict for Central Europe hot and dry summers and an increase of short and intense rainfall [1], which will cause a spatial shift of plant species based on species-specific sensitivity to environmental conditions. Beech is one of the most common deciduous trees in the forests of Central Europe [2]. Given the importance of the beech in the European forestry sector it is important to know its reaction to stress and ability to adapt to changing environmental conditions.Due to conflicting findings on the respond of beech to drought stress there is a need for specially focus study to trace the structures that adapt to changing environments base on the detail anatomical, physiological and molecular analysis. It is necessary to analyze all plant organs, because the roots, stems and leaves may differ in the sensitivity to drought. Many proteins are involved in the drought responses in trees [3]. Dehydrins (DHN) are desiccation-induced proteins [4] and are produced in response to dehydration during for example drought [5], low temperatures/freezing [6,7], or increased salinity [8], but also during seed maturation. Expression of DHN genes and other genes related to drought stress thus represent potential markers for identifying stressed trees [9].In this study, we will present the differences between drought stressed and normally developed beech seedlings and put the anatomical and physiological changes into the context of different levels of genes expression. Our main focus is on DHN genes and their expression during drought stress compared to the normal irrigation. In order to discover the link between anatomical and physiological changes and DHN expression we decided to run transcriptome analysis of beech. We detected the DHN's and DHN-like proteins in the transcriptomic data. Main goals of our study are to (i) compare the relative DHN expression of drought stress and control seedlings; (ii) identify potential drought stress markers and to (iii) design q-PCR probes for DHN without the need of running time and money consuming RACE-PCR.This work was funded by Mendel University in Brno (Grant IGA 73/2013 and LDF_VP_2015034/2015).

Poster number : S4.2

Analysis of genetic diversity and population structure in Calamus thwaitesii Becc. using microsatellite markers

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Calamus thwaitesii Becc. (Arecaceae) is one of the most widely distributed rattan palms in south India. The natural populations of the species are getting depleted rapidly due to indiscriminate collection mature canes for the furniture industry. Adequate re-planting or cultivation practices are not followed in many places. A detailed analysis of genetic diversity and phylogeny was carried outfir the first time using thirty-two microsatellites in forty-two samples. Out of the microsatellite loci tested, twenty-three were polymorphic (72%). The individuals varied greatly as evidenced from different diversity estimates. The number of alleles per locus ranged from 1.09 to 1.41, Shannon Information Index as a

measure of the genetic diversity ranged from 0.06 to 0.28, average observed heterozygosity from 0.09 to 0.41, expected heterozygosity from 0.04 to 0.20 and polymorphic loci ranged from 8.70% to 39.13%. Considerable genetic differentiation (Fst) was found between the populations and the level of theoretical gene flow was estimated at 0•32, indicating lower migration rate between populations of the C. thwaitesii. Average Fst of 0.58 was observed in this study, indicating that 58% of the microsatellite variation in the C. thwaitesii populations was found among populations. A dendrogram was constructed using genetic distance values which showed many groups which were clustered not in accordance with geographic locations. To complement the results obtained using the cluster analysis, inference on population admixture was obtained using the STRUCTURE software. Though K = 3 did not revealed any structure, in other runs at K = 4 to 11 levels showed various levels of structuring. In general, locations 1 (Rosemala), 2 (Bonacaud), 3 (Pandimotta), and 4 (Agastyamala) showed more diversity and hence structuring within the locations in comparison to 9 (Kalaketty), 10 (Silent Valley), and 11 (Subramanya). The present study generated valuable information shedding light on the genetic diversity, population structure, gene flow and phylogenetic parameters of C. thwaitesii.

Poster number : S4.3

Genetic diversity of Casuarina equisetifolia

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Casuarina equisetifolia ssp. equisetifolia, nitrogen fixation trees, natural distributed in subtropical and tropical coastal areas from northern Queensland and Northern Territory in Australia, throughout southern Thailand, Malaysia, Indonesia, the Philippines, Melanesia, Polynesia and Guam, etc. The EST-SSR markers were used for to determine the genetic diversity and population structure among the 29 typical natural and introduced populations, to offer basic information for germplasm collection, protection, selection, and genetic improvement and breeding project. Based on the 34,752 EST sequences of casuarina trees in the NCBI website untill April, 2015, the 367 SSR loci can be identified from the 12,063 UniGene, distributed in the 353 EST sequences, only 2.93% frequency contained SSR loci. The 13 pairs of EST-SSR primers with amplified stability, clear band and higher polymorphism were obtained and used for genetic diversity analysis. The 308 alleles can be identified from the 13 SSR loci, average alleles number per loci was 23.69, range of alleles number was from 11 to 48. Range of effective alleles number, Shannon's index, observed heterozygosity and effective heterozygosity were 1.533 - 7.029, 0.691 - 2.139, 0.270 - 0.655 and 0.393 - 0.858, respectively. According Shannon's index, the order of genetic diversity level from high to low of the 5 regions was: African introducted (AF) > Asia natural (AN) > Oceania natural (OP) > Central American introducted (CI) > Asia introducted (AI) ; the order of genetic diversity level of the 29 populations was given. The results implied that serious inbreeding between populations were occurred during the whole distribution. The main variation of C. equisetifolia populations were from the individuals within populations, which accounting for 70.12% of total variance. On regions level, the order of variance was: AN (81.15%) > AI (74.58%) > CI (72.29%) > AF (68.43%) > OP (61.45%). Results showed that family selection among population should be the focus of breeding. Meanwhile, though variation from populations accounted for only 25.42 % to 38.49% of the total variation, given the serious inbreeding that identified in the population, population selection should also attach great importance in future. Based on UPGMA dendrogram of 29 populations of C. equisetifolia using Nei's unbiased genetic distance, proved that introduced populations of China should be from Asia natural populations, while introduced populations of Kenya, and India and Veitnam might from Oceania natural populations.

Poster number : S4.4

Multidirectional gene flow leads to intricate reticulate evolution in box eucalypts

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Reticulate evolution by hybridization is considered a common process that shapes plant evolution. However, its frequency and magnitude has received little attention. Box eucalypt species (series Moluccanae) provide an ideal system to approach questions about the multidirectional patterns of gene flow in recently evolved groups. They form a diverse and phylogenetically undefined group with overlapping morphological features and potentially extensive hybridization. Moreover, this group is ecologically important given its dominance in Australian grassy woodlands that have suffered from clearing for other land use, and therefore of strong conservation interest. We used a multi-faceted approach, combining analyses of chloroplast and nuclear DNA, as well as seedling morphology, flowering time records and ecological space differentiation in order to test for monophyly and the influence and magnitude of regional hybridization in the reticulate evolution of this group. Reticulated patterns could also be due to incomplete lineage sorting which is difficult to discriminate from hybridization. Under hybridization we expected that sympatric populations of different species would be genetically more closely related than distantly located populations of the same species. Furthermore, species with small population sizes were expected to be better resolved than species with larger population sizes if lineage sorting was complete. The different layers of information were consistent and suggested a lack of monophyly at different hierarchical levels due to multidirectional gene flow among several species. Therefore we used geography and population sizes to distinguish between these two processes. We found that chloroplast and nuclear DNA were shared among different species in geographic proximity, consistent with the hypotheses about geographic introgression zones in Eucalyptus. Species with smaller population sizes appeared better resolved. Our study highlights that the delimitation of box eucalypt species requires the combination of molecular approaches with morphological characterization, distribution and flowering time, and suggests that hybridization is a recurrent process in the evolution of this ecologically important group of Eucalyptus.

Poster number : S4.5

Variation and genotyping of SNP marker based on nine drought-responsive genes in Populus spp.

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Drought is one of the most common abiotic stresses responsible for yield losses in commercial tree species. Single nucleotide polymorphism (SNP) markers are very useful tools for population genetic studies and genotype/phenotype association mapping. A large amount of sequence data for many functional genes has been obtained from the genome projects for poplar, aspen, cottonwood and their interspecific hybrids. A total of 346 SNPs from the re-sequencing of partial genomic sequences of nine drought-responsive genes were discovered in genus Populus. The nine drought-responsive genes are dehydrin, alternative oxidase 1a, amino acid permease I, galactinol synthase, transcription factor like protein, ethylene-responsive element-binding factor 1, 4-hydroxyphenylpyruvate dioxygenase, leucoanthocyanidin dioxygenase-like protein and putative Zinc finger protein. From the SNPs, we developed the 57 panels of SNP genotyping for detecting the drought linked genes with TaqMan probe-based real-time PCR. The panels successfully generated a total of 155 SNP types for 100 samples of P. davidiana, P. glandulosa, P. alba, P. alba x glandulosa, P. maximowizii, P. nigra, P. deltoids and P. euramericana. Seventy-eight haplotypes were reconstructed from the SNP typing of nine genes over the poplar species using an EM algorithm function implemented in the PowerMarker v.3.25 program. The number of haplotypes varied in genes and species with an average of 8.67 haplotypes per gene. Species-specific haplotypes were also found in P. davidiana and P. maximowiczii. The panels were then applied to 58 samples from five natural populations of P. davidiana in South Korea. The averages of the number of alleles per gene and the observed heterozygosity were 5.33 and 0.661, respectively. The Wright's F-statisitics were 0.2601, -0.1007 and 0.1317 in the populations. The results indicated that high level of genetic variation and considerable genetic differentiation among the populations of P. davidiana were existed for the nine drought-

responsive genes in South Korea. The SNP assays developed from the drought-related genes would contribute to population genetics and genotype/phenotype association mapping studies in poplar species.

Poster number : S4.6

Genetic Diversity and Population Stucture of E. globulus

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Eucalyptus globulus sp. globulus Labill (Tasmanian Blue Gum) is a native species from southeast Australia. Because of its commercial value, in the 80's a seed collection was gathered and considered representative of the species natural diversity throughout its area of distribution. Using this collection, and 35 quantitative traits such as flowering, growth and leaf morphology, it was determined that E. globulus presents a population structure of 13 races and 20 sub-races (Dutkowski and Potts 1999).A detailed knowledge of the population structure of a species is a key factor to understand the evolutionary history of populations. This knowledge has direct impact in species management at an ecological or conservationist level, in the implementation of genetic improvement programs and in the development of association studies between genes and traits.Sixteen populations representative of E. globulus natural diversity in Australia (398 samples) were genotyped with 24 SSR, in order to verify if it was possible to detect significant genetic differences in populations from 16 different geographic regions, throughout the entire Australian area of the species natural distribution. The results show that the 16 Australian populations are genetically diverse and differentiated. Nine clusters/"races" were detected and the results do not deviate much from those reported in previous studies based on quantitative traits.With this framework data, we are able to investigate the provenance of individuals of unknown pedigree and assess the levels of representativeness of E. globulus natural variation in different populations. Moreover, new individuals, with specific traits (e.g. improved adaptation, pest tolerance, wood properties, early flowering), can be selected and introduced in breeding populations enriching the genetic pool of ongoing genetic improvement programs.

Poster number : S4.7

Lineage-specific and adaptive expansions and function diversification of subgroup 4 R2R3-MYB transcription factors during the evolution of conifers

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MYB proteins are one of the largest and functionally most diverse groups of transcription factors in eukaryotes. In higher plants, distinctive members of this family have selectively expanded and regulate a variety of plant-specific processes. These include the secondary wall formation and the phenylpropanoid metabolism, which are in part governed by the R2R3-MYB family. A subgroup of this family (subgroup 4) is especially important in conifers, where they orchestrate responses to drought, wounding and other environmental stimuli. Taking advantage of the large amount of conifer sequencing and expression data that has accumulated in recent years, we conducted a series of tests for natural selection in a phylogenetic context for this subgroup of R2R3-MYBs. We initially built a large database of ~300 conifer and angiosperm sequences of MYBs from subgroup 4 and other related clades (i.e. subgroups 5, 7 and WPS II and III), and constructed a phylogenetic tree under a Bayesian framework after a rigorous codon alignment. Subgroup 4 MYBs were clearly separated from others subgroups. In this subgroup, angiosperm MYBs were also split from those of conifers, and conifer genes were distributed among three different clades: one (conifer I) that was specific to the

Pinaceae, a sister group (conifer II) containing only non-Pinaceae sequences, and a third one (conifer III; at more basal position) with sequences from various conifer families from both Pinaceae and non Pinaceae taxa. While all three conifer groups harboured footprints of purifying selection, a significantly higher evolutionary rate was observed in the conifer II clade, potentially indicative of relaxed selection and/or increased mutation rates in these sequences. The Pinaceae clade was further divided in five groups. All of them included paralogs of different genera, but one of these clades exhibited increased evolutionary rates in Abies and two others in Pinus and Picea sequences. Comparisons with published linkage maps for Picea glauca additionally revealed a significant correlation between phylogenetic and linkage groups, with all members of subgroup 4 mapping on linkage group 7, thus suggesting limited translocation since duplications. A further positive (although non-significant) relationship between map and phylogenetic distances was hinted, while there was a significant and negative correlation between map distance and similarity of expression profiles. Our results thus suggest an expansion of subgroup 4 R2R3-MYBs in conifers, with differing patterns of evolution across families. Diversification within the Pinaceae was confined to specific regions of the genome and involved a relatively rapid functional divergence that supports a hypothesis of compartmentalization.

Poster number : S4.8

A complex evolutionary history shaped the distribution of Abies alba (Mill.) genetic variation along the Apennines

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Silver fir (Abies alba Mill.)is one of the most important forest tree species in Europe. Palaeobotanical and genetic studies have generated contrasting hypotheses about its dynamics within the Quaternary period. Among others, unclear topics are the distinction between isolated and effective refugia, their location, and the temporal scale and intensity of gene exchanges between refugial areas.In the Apennines, silver fir presence during the Late Glacial has been demonstrated by local palynological surveys. The existence of at least two different genetic clusters has been hypothesized based on a limited number of sampled populations, mostly located in Calabria and northern Apennines, with large geographic gaps. Anthropogenic pressure and climatic events have been described as the main drivers of silver fir rarefaction in the Apennines, with intense debate about what would have been the most important process. The result of these two processes is that nowadays silver fir populations are highly fragmented, with some stands reduced to a few hundred individuals, often highly isolated from surrounding populations.This work aimed at investigating the genetic structure of Apennine populations to reconstruct their past demographic and recolonization dynamics. We sampled the entire Apennine range, focusing on previously unsampled areas and including, for comparison, populations from surrounding biogeographical regions (i.e. the Alps and Eastern Europe). All individuals were genotyped with nuclear and chloroplast microsatellites. The intensive sampling scheme and the large marker set used allowed to deeply investigate: i) the genetic relationship between northern and southern Apennine populations, ii) the origin of populations from central Apennines, iii) the possible presence of contact zones and genetic discontinuities along the Apennine range, and iv) the genetic relationship between Apennine gene pools and surrounding ones.The 1167 sampled individuals were grouped by Bayesian clustering techniques in two main genetic clusters, separating populations north and south of the Gran Sasso and Majella massifs (~42.5 N). Populations from northern Apennines were more similar to alpine populations than the ones from southern Apennines, which unexpectedly clustered with Balkan populations, supporting the hypothesis of a relevant role of pollen gene flow in connecting these two distant areas. Furthermore, the distribution of within-population genetic variation along the Apennines highlighted that populations from the two areas where the species was more abundant during the Holocene (Northern and Southern Apennines) retained the highest genetic variation, whereas lower genetic variation occurred in central Apennines and in the Alps.

Poster number : S4.9

Phylogenomics of tropical Asian forest trees

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Tropical forest ecosystems are amongst the most species-rich habitat on the planet, providing ecosystem services to millions of people, serving as shelter for thousands of unique plants and animals, and acting as important climate regulators. Evolutionary and biogeographical studies of species-rich tropical forest groups are hampered, however, by their extensive geographic ranges, a general paucity of diagnostic morphological characters and limited success of previous studies due to the traditional use of limited, low-informative molecular markers. This has resulted in fragmented knowledge on the evolutionary history, genetic diversity and ecological functioning of key plant families of tropical ecosystems, while we are faced with a global loss of biodiversity due to unsustainable use of biological resources, climate change and deforestation.Here, we present preliminary results from our study, coupling the latest next-generation genomic sequencing techniques and recent advances in phylogenetics, molecular dating and biogeographical reconstruction analyses. Our aim is to unravel the origin, evolutionary diversification, historical biogeography and causes for genomic diversity in three economically and ecologically dominant plant families in the Sino-Australasian region.We are in the process of establishing genomic resources for three of the largest tree families found in the subtropical and tropical rainforests of the Sino-Australasian region. The initial work on a representative species dataset for Sino-Australasian Lauraceae, (200 species), Fagaceae (400 species) and Dipterocarpaceae (200 species) is presented, showing sampling from former continental fragments, islands and tropical mountains to incorporate as much evolutionary information as possible.Using next generation sequencing methods, we produced one gigabase of genomic sequence for each species, assembling complete chloroplasts and nearly complete nuclear ribosomal cistrons for selected taxa and outgroups. We then used both datasets to perform phylogenetic analyses, molecular dating and biogeographical reconstruction to evaluate patterns of diversification and genomic divergence in the three families. Genomic diversity was compared using sliding window approaches and intergeneric comparisons amongst the three families.We expect that the results of our study will initiate a better understanding of geographic distribution of genomic diversity and it's evolutionary causes, within Sin-Australasian rainforest trees, and their evolutionary connections to surrounding regions. In the face of overwhelming evidence for an anthropogenic biodiversity crisis, this improved understanding will inform management strategies for the tropical forest biome across Asia, both from the point of economical as well as conservation perspectives.

Poster number : S4.10

On genetic characterization of Norway spruce (Picea abies L.) on the NW Balkan Peninsula using three types of markers – preliminary results

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Genetic diversity and structure of Norway spruce populations across Europe is relatively well described with the exception of parts of the Balkan Peninsula. Here glacial refugia have been stipulated to exist. In the presented work we combine results obtained with isoenzyme, mitochondrial DNA and microsatellite analysis to describe genetic diversity, structure and migration paths in the NW Balkan Peninsula. We include the results in the wider European context.

Poster number : S4.11

Phylogenetic analyses of two varieties of genus Thujopsis, Cupressaceae (sensu lat.) based on EST-SSR polymorphism

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The Cupressaceae clade has the broadest diversity of habitats and morphologies of any conifer family, and the genus Thujopsis, which belongs the Cupressaceae, is considered as one of the early-diverging genera (Pittermann et al., 2012). Thujopsis is native to Japan and includes two varieties, Thujopsis dolabrata Sieb. et Zucc. as a southern variety and Thujopsis dolabrata var. hondae Makino as a northern variety (Hayashi, 1960). This genus has a strong shade-tolerance ecologically (Hitsuma et al., 2015), and is an important tree species in Japanese forestry because of its superior wood properties (Inamori et al., 2006). However, there have been few reports about genetic differences between the two varieties. In this research, we have developed expressed sequence tag-simple sequence repeat (EST-SSR) markers from Thujopsis dolabrata var. hondae Makino (Sato et al., 2015), and investigated the genetic diversity and phylogenetics of the genus Thujopsis.Phylogenetic tree based on EST-SSR data strongly supported the monophyly of genus Thujopsis and the two varieties. Within the Thujopsis dolabrata, a clade composed of northern populations located in the central part of Honshu Island is sister to one composed of the remainder of the variety. Within Thujopsis dolabrata var. hondae Makino, two isolated populations located in southern marginal area of natural distribution of the variety formed a well-supported (99%) basal group, and is sister to one composed of the remainder. We conclude that major clades in the phylogenetic tree of genus Thujopsis reflected to biogeographic pattern of two varieties.

SESSION 5

Poster number : S5.1

Bridging science and community: Re-introduction of Dacridium nausoriense - an endemic timber species

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Recent work on the genetic analysis of Dacridium species have identified that the species in remote Oceania is a monophyletic group that arose and diversified within the last 20 million years through long-distance dispersal and a range of speciation mechanisms. Dacridium spp. population in Fiji is threatened due to over-exploitation for timber and other uses. Fiji has two Dacrydium species namely Dacridium nidulum and Dacridium nausoriense; that differ in adult-leaf and pollen-cone size. D. nausoriense is endemic and also classified as Endangered. The main objective of this presentation is to highlight how the information gathered from genomic analysis is used to bridge science and communities through transfer of knowledge, awareness and re-introduction of this endangered species in its natural habitat. This presentation will also highlight preliminary findings on the vegetative propagation technique used for mass production of planting materials. These efforts will ensure that all relevant stakeholders are involved and thus would allow the sustainable management and conservation of this endangered species.

Poster number : S5.2

Genomic Selection in Douglas-fir (Pseudotsuga menziesii)

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Genomic selection (GS) can offer unprecedented gains to forest tree breeding, especially for late expressing traits and those with low heritability. Here, we used: 1) exome capture as a genotyping platform for 1,348 Douglas-fir trees representing 42 full-sib families growing on 3 sites in British Columbia, Canada and 2) growth and wood quality traits as phenotypes. Two GS predictive methods (Ridge Regression Best Linear Unbiased Predictor (RR-BLUP), and Generalized Ridge Regression (GRR)) were used to assess their predictive accuracies over space (within site, cross sites, and multi-site to single site) and time (age-age). Additionally, an independent validation population consisting of 490 individuals were used to verify the original 1,348-tree models. The RR-BLUP and GRR models produced similar predictive accuracies across all traits. Within-site GS prediction accuracies were high and generally better than the combined sites and multi-site to single-site predictive accuracy fell in between these two values. Cross-site predictions had the lowest predictive accuracy, reflecting genotype x environment interactions. The independent validation produced poor predictive accuracies, most likely due to the breakdown of linkage disequilibrium over successive generations, leading to the inability of the derived models to produce reliable predictions and ultimately confirming the importance of regular GS models update in response to the changing genetic architecture of the population as a whole over multiple generations.

Poster number : S5.3

In vitro seed germination Of Pycnanthus angolensis (Welw) and Zanthoxylum zanthoxyloides (Lam.)

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Pycnanthus angolensis (Welw.) and Zanthoxylum zanthoxyloides (Lam.) are tree species with great importance in traditional ethnomedicine. However, these species are threatened by extinction arising from pressure on their genetic diversity, over-utilisation and difficulty of their propagation through conventional methods. Therefore, effect of priming using water and different growth media on germination of P. angolensis and Z. zanthoxyloides seeds were investigated.Two hundred and forty mature fruits of P. angolensis and Z. zanthoxyloides were collected from Nigeria. One hundred and twenty seeds of each species were soaked in deionised water for 24 hours while the rest were not. Seeds were sterilized with 70% ethanol for two minutes and 50% Clorox for five minutes before inoculation. Murashige and Skoog (MS), woody plant medium (WPM) supplemented with 10 mg/L Indole Butyric Acid (IBA), 0.5-5.0 mg/L 6-Benzyl Amino Purine (BAP), and 5.0 mg/L kinetin (KIN) were combined for in vitro seed germination. Cultured bottles were arranged in Completely Randomised Design (CRD) and replicated four times. The setup was observed fortnightly to determine the onset of germination. Data were analysed using descriptive statistics and ANOVA at α=0.05. Priming of seeds in deionized water enhanced germination of P. angolensis seeds. Germination at 14.00±16.24 days were observed in soaked seeds inoculated on WPM+ 5.0 mg/L BAP+ 5.0 mg/L NAA while Z. zanthoxyloides did not germinate. Germination percentage, 62.50±43.30% was observed on MS+ 2.0 mg/L BAP+0.5 mg/L NAA+10.0 mg/L IBA. Longest root length of 1.88±1.32 cm was recorded for P. angolensis at 6WAP on pure MS. Media type with different hormonal combinations affected the two species. Germination of P. angolensis seeds inoculated in WPM+2.0 mg/L BAP+ 0.5 mg/L NAA+10.0 mg/L IBA started after 7 days. WPM+5.0 mg/L BAP+5.0 mg/L NAA had germination percentage of 56.3% and highest root length, 1.18 cm was observed in MS+2.0 mg/L BAP+0.5 mg/L NAA+10.0 mg/L IBA. Onset of germination in Z. zanthoxyloides seeds was recorded in WPM+2.0 mg/L BAP+0.5 mg/L NAA+10.0 mg/L IBA at 37 days. Germination percentage of 43.8% was recorded in MS+5.0 mg/L BAP+5mg/l KIN+10 mg/L IBA, while highest mean root length 0.80 cm was observed in MS+5.0 mg/L BAP+5.0 mg/L KIN+10 mg/L IBA. In vitro seed germination of Pycnanthus angolensis requires priming with water which enhanced germination. MS+2.0 mg/L BAP+0.5 mg/L NAA+10.0 mg/L IBA is recommended for in vitro germination of P. angolensis while MS+5.0 mg/L BAP+5.0 mg/L KIN+10.0 mg/L IBA is recommended for Zanthoxylum zanthoxyloides.

Poster number : S5.4

Next-generation transcriptome assembly of an Amazon palm (Euterpe precatoria)

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Euterpe precatoria is a palm species of the Arecaceae family. The species occurs in the northwest and central regions of the Amazon rainforest, and it grows preferably on well-drained and low fertility soils. Euterpe precatoria produces açai fruits that are processed and consumed as smoothie, jelly, juice, candies and ice cream. Additionally, it produces a palm heart that can be consumed. Natural populations are threatened by predatory exploitation and deforestation in the Amazon. With the advent of next-generation sequencing technologies (RNAseq), the genetic diversity of many forest species, as well as their evolutionary processes, can be better understood. Here we describe the generation of a reference transcriptome for E. precatoria using RNAseq, developed to support population and genetic studies. Leaves of one adult individual were collected in the Amazon rainforest (Brazil), and immediately frozen in liquid nitrogen and lyophilized. Total RNA was isolated and cDNA libraries were sequenced with the Illumina NextSeq platform. A total of 95,232,362 raw reads (paired-end reads of 151 bp length) were filtered by quality with Trimmomatic and assembled into 241,205 transcripts with Trinity. The E. precatoria de novo transcriptome assembly contains 201,545 unigenes represented by 86 Mbp, with a median (mean) contig length of 282 bp (359 bp) and a GC content of 44.76%. Unigenes were annotated for their putative functions based on the Arabidopsis thaliana transcriptome database. A total of 12,575 annotated unigenes were categorized into 31 functional groups under Gene Ontology terms. In the biological process category, cellular processes (41.07%) and metabolic processes (37.84%) were the predominant groups. For cellular component category the predominant were cell part (53.64%) and organelle (34.02%). The main distributions in the molecular function category were catalytic activity (38.79%) and binding (36.39%). The E. precatoria reference transcriptome was also analyzed for the identification of simple sequence repeat (SSR) markers. A total of 5,099 SSRs were identified along the transcriptome using 10,4,4,4,4,4 motifs repeats criteria for mono-, di-, tri-, tetra-, penta-, and hexa-nucleotides SSRs. Dinucleotide repeats were the most abundant type of repeat, representing 59.15% of the total. Trinucleotide repeats constituted roughly 20.48% of all the SSRs detected. The most common dinucleotide motif was AG/CT-GA/TC, and corresponded to 47.84% of the 3,016 SSRs identified in this category. This transcriptome represents a valuable genomic resource for E. precatoria that will be used for future research on genetic diversity, evolution and breeding for this species.

Poster number : S5.5

Transcriptome analysis of Euterpe edulis and identification of microsatellite markers

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Euterpe edulis is a palm species from the Arecaceae family native to the Brazilian Atlantic forest. It is a plant that prefers shady and humid environments, and plays a very important role in forest dynamics. It produces an abundance of fruits that serve as food for many species of wildlife. The palm heart is the main exploited product. The growing demand for that product in Brazil is driving this species to local extinction. The genetic characterization of E. edulis is indispensable to propose management and conservation strategies for the remaining natural population. With the objective of developing genomic resources for this species, total RNA was isolated from leaves of one adult individual of E. edulis, converted into cDNA, and used to prepare sequencing libraries. The Illumina NextSeq platform was used to produce transcriptome sequences. A total of 81,724,584 raw reads (paired-end read of 151 length) were filtered by quality with Trimmomatic and assembled into 288,275 transcripts with Trinity. The E. edulis de novo transcriptome assembly contains 235,419 unigenes represented by 120 Mbp, with a median contig length of 292 bp, mean contig length of 372 bp, and a GC content of 45.21%. Of all unigenes, 8,428 were functionally annotated to one or more Gene Ontology categories based on Arabidopsis thaliana. Two predominant groups in the biological process category were cellular (40.97%) and metabolic processes (37.87%). For the cellular component category the predominant groups were cell structure (52.39%) and organelle (32.57%). The main distributions in the molecular function category were catalytic activity (39.48%) and binding (36.47%). Beyond SNPs, we can also developed microsatellites from transcriptomes. About 12,346 sequences were examined, anda total of

2,724 microsatellites were identified based on 10,6,5,5,4,4 motif repeats criteria, according to the number of sequences that contain mono-, di-, tri-, tetra-, penta-, and hexa-nucleotides. The number of sequences containing more than one microsatellite was 365. Trinucleotide and dinucleotide were more abundant, respectively representing 31.41% and 14.01% of the total SSRs found. The trinucleotide motif AAG/CTT was most represented in this category (23.87%). Potential marker sites identified will be selected, validated, and used to develop EST-SSR primers for this species. Here we present a broad survey of the E. edulis transcriptome, as well as an extensive search for molecular markers that may be applied to analyze the genetic diversity of E. edulis natural populations and germplasm bank.

Poster number : S5.6

Draft Genome and Gene Annotation of Lentinula edodes

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Lentinula edodes, called the shiitake mushroom, is one of the most important and popular cultivated edible mushroom with its various utilities as foods and medicinal fields. Here, we represent the 46.1 Mb genome of L. edodes, encoding 13,028 predicted gene models. The genome assembly consists of 31 scaffolds and with 0.45% N's (N50, 3.66 Mb; N90, 0.81 Mb, the longest scaffold, 5.85 Mb). Gene annotation provides key information for various signaling pathways and secondary metabolites. This genomic information would contribute to establish the basis for developing molecular genetic markers for MAS/MAB and increasing our understanding of the genomic structure and functions.

Poster number : S5.7

Assessing the utility of genomic selection in Eucalyptus breeding

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Genomic Selection (GS) is a breeding methodology that can increase genetic gains by accelerating the breeding cycle or by improving the selection accuracy. We used 44,250 SNPs scored in a Eucalyptus population comprised of 86 E.grandis, 82 E.urophylla, and their 949 F1 hybrids to develop GS models for four phenotypic traits, basic density, pulp yield, and volume in trees scored at three and six years of age. The model prediction accuracies were optimized by considering the effects of three aspects, (1) statistical algorithms, (2) selection of training set, and (3) SNP subsets. Four statistical algorithms were applied including ridge regression-best linear unbiased prediction (rrBLUP), GBLUP, Bayesian LASSO, and reproducing kernel Hilbert spaces (RKHS). The GS models using rrBLUP, GBLUP and Bayesian LASSO performed similarly well in predicting all the traits. The GS models were found to outperform the conventional pedigree method in predicting traits. We evaluated the impact of four different training sets considering genetic relationship between training and test sets and genetic variance between them. The GS models using the training set that was comprised of all parents and partial F1 progenies gave the best performance in all traits. The average predictive ability across training sets for the rrBLUP algorithm was 0.44 for pulp yield, 0.5 for basic density, 0.18 and 0.32 for volume at 3 and 6 years old, respectively. Furthermore, analyses using subsets of SNPs suggested that using 20k markers is sufficient for GS in our hybrid (E.grandis x E.urophylla) breeding material. Our results suggest that GS model could be applied as a valuable tool for improving Eucalyptus breeding efficiency.

Poster number : S5.8

Genomic Selection and Genome wide Association Studies in Eucalyptus urophilla x E.grandis

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Eucalyptus species have high growth rates and the ability to a broad range of geographic locations. Their wood is used as a raw material for the production of cellulose. In general, traditional Eucalyptus breeding takes over 12 years to select plus trees from seeds. Hence, genomic selection (GS) using single nucleotide polymorphisms (SNPs) analysis would be useful breeding tool to shorten terms for elite tree selection. In this study, we apply SNPs analysis for GS into conventional breeding of Eucalyptus in tropical and subtropical regions of Brazil. Moreover, we identified genes involved in each phenotype by genome wide association studies (GWAS). The data of phenotypes and genotypes were obtained from 931 three-year-old hybrid Eucalyptustrees grown in the plantation of AMCEL,MP, Brazil. They are composed of more than 50 families, and 6-30 trees from each family were planted. We measured growth rates (height and diameter) and wood properties (hemicellulose, alpha-cellulose and Klason lignin contents) by using Near-infrared spectrum analysis. We used 25K SNPs selected from EuChip60K project1, and investigated correlation between phenotypes and genotypes of 931 trees. In GS, we used two analytical approaches, such as rrBLUP (best linear unbiased prediction) and BayesB method for prediction model. GWAS was carried out by Plink. The accuracies of the model varied from 0.13 of hemicellulose contents to 0.30 of basic density by rrBLUP method. GWAS appeared several significant SNPs associated with wood volume traits. The most significant SNP with wood volume was in chalcone flavanone isomerase gene. These results are becoming an important data to practice phenotype prediction.

Poster number : S5.9

Accuracy of genomic prediction in a multi-generation population of maritime pine

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The maritime pine breeding program follows a recurrent selection scheme originally based on a population of 635 plus-trees (called G0 trees) selected during the 1960ies. Since then the main population has cycled through three generations (G0, G1 and G2). High throughput genotypic technologies through genomic selection (GS) can be a paradigm shift to reduce selection cycle length, increase the selection intensity and manage genetic diversity along generations in conifer breeding.Given the low level of linkage disequilibrium and the low number of markers available (<5,000) to cover the large genome of maritime pine (24 Gb/C), we used two proof-of-concept experiments with contrasted population structures to explore key factors impacting GS accuracy. The first population had a large genetic base (NS ≈ 100) with two generations (G0 and G1). The second population had a small effective size (NS = 25) with three generations (G0, G1 and G2). For the first and the second population, 2,500 SNPs and 4,300 SNPs were available, respectively. Breeding values for growth and stem straightness obtained from classical genetic evolution of the whole breeding population (~400 000 individuals) were used as pseudo phenotypes in genomic prediction.For the first population, average prediction accuracies ranged from 0.43 to 0.56 depending on the trait. For the second population, using the parental generation (G0 and G1) as the calibration set and progeny population (G2) as the validation set, GS produced higher prediction accuracies with a range of 0.70-0.85. The study highlighted the effect of population structure (size and relatedness) on genomic prediction accuracy. Even with low marker coverage, genomic selection could be efficient in maritime pine especially with low effective population size. We proved for the first time in a conifer that a model trained on one generation (G0 and G1) can be used to predict progeny phenotype with high

accuracies. This is likely due to efficiency of markers tracing segregating haplotypes in progeny population. We currently combine information from both designs in a single-step approach, using a hybrid matrix (pedigree and markers) for genetic evaluation of larger number of individuals. This study will use all molecular information available and will be a first step in the integration of genomic information for the genetic evaluation of individuals.

Poster number : S5.10

Preliminary training of a genomic evaluation in a breeding pedigree of Populus nigra L.

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Genome-wide evaluation (GWE) is expected to bring substantial benefits to the genetic improvement of perennials like poplar. Firstly, because current phenotypic evaluation is lengthy, costly and complex for the species. Secondly, because identifying poplar clonal varieties rely on a costly built-up of large segregation families from which to draw the best candidates combining favourable alleles with additive and non-additive effects.The present study provide preliminary assessments of the feasibility of GWE in a highly monitored subset of the French black poplar breeding pedigree. The training subset comprised a factorial mating design involving 6 parents and 1 known grand-parents and 261 individual records. Recorded traits were growth in the form of tree circumference and height, and architecture assessments taken as branch angle. Patterns of environmental heterogeneity in the trials were carefully taken into account at the individual tree level, by implementing autoregressive and spline-based two-dimensional smoothing surfaces into the evaluation model. All individuals have been genotyped thanks to a recently developed 12K SNP chip, which produced in our pedigree 7812 usable SNPs. Missing genotyping values, which amounted to 1.7% of data, were imputed by the FImpute software. Several genomic best linear unbiased predictors (G-BLUP) models were applied using GS3 software.Results provided prediction abilities for each trait obtained from a cross-validation approach. Accuracies of these predictions differ according to architecture and heritability of these traits. The benefits of imputation and environmental heterogeneity adjustments are discussed at the light of these results, together with the impacts that a GWE based selection would have on the relatedness among candidates compared to a pedigree-based approach.

Poster number : S5.11

SNP development and genetic diversity of the non-native tree Robinia Pseudoacacia in Belgium

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For as long as humans have travelled, species have been transported, deliberately or inadvertently, from their native ranges to new areas. Non-native tree species are introduced into new natural ecosystems mainly for production or ornamental aims. In this study we focus on Robinia pseudoacacia (Black locust) which is native of the eastern part of United State and was introduced in Europe at the beginning of the 17th century. Firstly used for its horticultural properties, interests in black locust became numerous for foresters and industrials. However, black locust is a nitrogen-fixing tree also able to reproduce and proliferate rapidly becoming a major environmental problem in many European countries. The aims of this study are to develop nuclear SNP molecular markers to investigate the genetic structure and differentiation among populations from the native range, from Belgium and from commercial Hungarian/Romanian orchards. Nuclear SNP markers were developed on this non-model tree species using RAD sequencing approach on 9 genotypes collected in various native and non-native populations. A total of 2020 RAD loci were subsequently analysed. Between them, more than one third was identified as potential paralogous, and was filtered accordingly. Finally, 336 SNP were validated by genotyping as suitable markers for population genetics applications. A total of 703 black

locust trees were sampled in 28 populations of the USA (13), Belgium (10), Hungary (3) and Romania (2), and were genotyped with 139 SNPs. Probability of identity was of 10-31 and clonal genotypes were detected with a maximum of 197m between two clones, indicating a high capacity of vegetative reproduction across long distances. Four private alleles were detected in native range specific to one or two populations and were identified in all European populations. The diversity was of He = 0.256 for North American populations, He= 0.274 for Belgian populations and He= 0.279 for cultivated black locust from Hungary and Romania. A strong genetic differentiation was observed between the native range and Europe, but not between Belgium and commercial orchards. This suggests a separate evolution of American and European populations, and an important impact of commercial resources on the species diversity in Belgium.

Poster number : S5.12

Genetic linkage mapping using full-sib progeny popualtion of Populus davidiana Dode

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Genome projects of tree species have been accomplished for poplar and spruce, while many of other tree species including loblolly pine have been processing in the World. In South Korea, Forest Resources Genome Project was recently initiated. It aims at completing whole genome draft sequencing and developing various markers of Populus davidiana, Pinus densiflora and Castanea crenata, which can subsequently enhance the industrial values of forest resources in terms of climate change, renewable bio-energy, genetic diversity, and new variety development. The immediate goals of the project are to establish populations for genome-assisted breeding, to construct genome sequencing of the three species, to develop molecular markers using comparative genomics and integrative omics approaches and to construct forest bioinformatic center for user-centric utilization service. In this study, the construction of the genetic linkage map of P. davidiana is mainly presented. P. davidiana Dode is one of the native poplar species and distributed widely throughout South Korea. It grows well on hillside and mountainous areas and thus this species is considered as worth due to growth and survival ability at barren and dry soil. Framework of genetic linkage map of P. davidiana has been carried out for growth and drought traits, constructing foundation of genomic selection breeding and establishing physical map using Next Generation Sequencing. We made artificial crossing between SN4 (female) and PG4 (male) that were selected as superior clones for volume growth based on genetic tests. A total of 64 progenies were successfully survived at field. At age 12, leaf samples from all progenies were collected and parents were also sampled. We applied an efficient sequencing method; the genotyping-by-sequencing (GBS) to construct the genetic linkage map. Thegenomic DNAs were extracted from leaves by Qiazen™ plant mini kit. The DNA was cut with two kinds of restriction enzymes and the GBS libraries had been constructed. At the moment, the sequencing was being carried out. By end of May, we expect that a saturated genetic linkage map for P. davidiana could be constructed on nineteen linkage groups and GBS-SNP markers would be developed for marker-assisted breeding. We also have a plan to study for physical mapping on the basis of linkage map and fluorescence in-situ hybridization.

Poster number : S5.13

Using RAD sequencing to identify cpDNA polymorphisms in Fagus sylvatica.

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Intraspecific polymorphism of chloroplast DNA (cpDNA) appeared to be useful for studying phylogeography of several angiosperm tree species enabling the inference of their postglacial colonization routes.. However, the inferences on phylogeography of common beech (Fagus sylvatica L.) appeared to be more difficult, because most of cpDNA diversity appeared to be retained in southern Europe, with little structure found in Central Europe. We attempted to identify additional cpDNA polymorphisms which would allow to detect differentiation among populations at a finer scale.We used restriction site-associated DNA sequencing approach (RAD) to detect novel cpDNA

SNP markers. We sampled 95 individuals from 53 European populations representing a broad range of the species distribution. Total genomic DNA was extracted from leaves and digested using PstI restriction enzyme (13 cut sites expected in the beech chloroplast genome). Resulting RAD sequences were sorted individually by barcode and filtered to remove reads with uncalled bases and the overall Phred quality score under 20. Reads were subsequently aligned to the Fagus sylvatica chloroplast genome using BWA v.0.6.2. SNP polymorphisms were detected and genotyped using GATK v.3.5. Only SNP polymorphisms with parameters QD (quality by depth) ≥6 and GQ (genotype quality) ≥20 were retained for haplotype analyses.Variant identification by GATK resulted in a total of 6 new polymorphic SNP loci; however, two pairs of loci appeared to be redundant (located within the same RAD reads). Finally, we retained 4 cpSNP loci, which showed differential polymorphism. Genetic diversity at individual loci ranges from 0.062 to 0.348, with a mean of 0.208. The four SNP loci generated six unique haplotypes. The overall haplotype diversity was found to be 0.637. The distribution of cpDNA variants across species distribution was not random. While the haplotypes H3 and H4 were quite homogeneously distributed in the studied area, the haplotypes H5 and H6 were common but exclusive of the Central Europe, but rare haplotypes H1 and H2 were present only in Central Europe. Using Mantel test we detected low, but significant correlation between genetic distance and spatial distance across sampled individuals (r=0.111; p=0.020). In the future we intend to resequence full-length cpDNA to find more polymorphisms enabling the detection of population differentiation at finer local scales.

Poster number : S5.14

Genetic diversity and reproductive patterns of Tilia cordata in Denmark – is the gene pool ready for a revival?

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Today Tilia cordata (Mill.) are only found in small, scattered and fragmented populations in Denmark, but earlier it was a dominating tree species (approx. 7000-500 BC). The species has only rarely been planted as it has not been of silvicultural interest and today is therefore commonly accepted as an indicator for ancient forest (Lawesson 1994). Tilia spp is an important species for biodiversity; it is one of the few insect pollinated large forest trees in Europe and it is important for many insects and fungi. Denmark is in the northern parts of the distribution range and viable seeds are only scarcely developed, but often it propagate itself vegetatively leading to the hypothesis that effective population sizes are considerably smaller than a simple count of number of trees would indicate.Due to the forecasted warmer future climate Tilia may again become a widespread species – but is the genetic base sufficient to ensure a satisfactory development of this species? In trying to answer this question seven populations have been sampled, four of which have been extensively or fully sampled in order to compare neighbouring trees and patterns of vegetative propagation. The seven populations have been chosen because they are, for the species in Denmark, large populations and are believed to be of natural origin. The sampled trees have then been analysed using 9 SSR markers developed for Tilia by Phuekvilai & Wolff (2013). Results show extensive clonal structures in three of the extensively sampled populations with genets as large as more than 20 ramets. In all of the sampled populations clones were detected. Estimates of effective population size will be presented and Tilia as an indicator of ancient forest will be discussed in relation to these new genetic results.

Poster number : S5.15

Genomic prediction in Cryptomeria japonica plus trees and evaluation of selection scheme combined genomic and phenotypic selections

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Cryptomeria japonica is one of the most important species for forestry in Japan. In order to reduce improvement period and to enhance prediction accuracy in this species, we performed genomic prediction of various traits (growth, wood properties, and male flower production) based on genotype and phenotype dataset of two different populations (constructed by 360 and 157 clones, respectively) of the first-generation plus tree clones. For genotyping of single nucleotide polymorphisms (SNPs), we used Axiom genotyping system based on a 70K SNP array designed for C. japonica. Phenotype data of the genotyped clones were obtained from clonal test sites with individual replications. We detected >31,000 SNPs were polymorphic and informative for the followed analyses. The accuracies of genomic prediction were different among traits and/or prediction models. Prediction for wood stiffness and male flower production productivity showed higher accuracy than that of the other traits. The accuracies were also different between the two populations. We calculated genetic gains of 20-year volume in some indirect selection schemes constructed by the combination of the genomic selection and early phenotypic selection. As a result, the combination of genomic selection of 20-year volume and phenotypic evaluation of 5-year height growth showed higher genetic gain among the selection schemes in the both two populations. We discussed the possibility of genomic selection for C. japonica improvement in the near future.

Poster number : S5.16

An assessment of DNA polymorphism among the oldest pedunculate aoks (Quercus robur L.) of Lithuania

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In Lithuania the pendunculate oak (*Quercus robur* L.), the 'king of trees', has a special place in culture and folklore and it is a symbol of strength and longevity. The largest areas of oak are located in the middle, southern and western part of Lithuania. In total 40.8 thousand ha of oak stands were registered in Lithuanian Statistical Yearbook of Forestry. The analysis of chloroplast DNA was done of 29 Lithuanian pedunculate oak populations (Pliūra *et.al*, 2009). The results revealed three distinct groups of populations in Lithuania, but the oldest oaks were not included in this research. Around the world, there are a lot of oaks which are famous in their country. In Lithuania 'Stelmužė' oak is the oldest and remarkable tree. It is believed to be at least 1.500 year old. This makes it the oldest oak in Lithuania and one of the oldest in Europe. 'Stelmužė' oak produce a small amount fruits, many of them are empty or damaged. Some lines were regenerated by application an embryo culture technique.
The main aim of this study was to assess the DNA polymorphism of 'Stelmužė'. It's offsprings regenerated in vitro and other oldest pedunculate oak individuals using RAPD molecular markers. Of the 6 studied primers, three produced no amplification bands at all, while the otherremaining primers amplified polymorphic products. Three informative primers Roth 370-10, Roth 370-06, Roth 170-09 were determinated. RAPD primers produced different number of DNA bands, and the size of amplified products ranged from 300 to 3000 bp. The highest number of bands resulted from amplification with the primers Roth 370-10 and Roth 170-09. The primers Roth 370-06 and Roth 170-09 revealed genotype-specific bands. One genotype-specific band for No. 21 (offspring of 'Stelmužė') was indentified with the primer Roth 170-09. The primer Roth 370-06 identified two genotype-specific bands for 'Stemužė' oak and 'Felinka' oak. The dendrogram showed the most closed genetic dinstance between two offsprings of 'Stelmužė' (No.15 and No.25). 'Stelmužė' oak (No.4) made cluster with 'Terpeikis' oak (No.28), as they grow in different places of Lithuania. A total of 50 RAPD bands were scored, of which 98 % were polymorphic. Obtained results conifrmed high genetic diversity among selected old oak individuals in geographically distict regions of Lithuanis and it gives an incentive to cintinue genomic research of old oaks by application different moleculatr markers.

Poster number : S5.17

Tracking SNP polymorphism in Polish pine stands for estimating of molecular variability.

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Commonly used microsatellite markers in molecular population studies, may cause some difficulties in analysis of Scots pine (Pinus sylvestris L.) population. A large and specific construction of genome in this species indicates that this type of markers can be a difficult challenge in the research. In our study we tried to implement a different method, relying on SNP (single nucleotide polymorphism), used as an alternative source of molecular variability. In our pilot analysis we started from sequencing of 12 genes (dhn1, dhn2, dhn3, dhn7, dhy-like, abaR, a3ip2, ccoamt, chcs, erd, dhn2PP, ln3-1) which were previously used in terms of nucleotide diversity pattern in local pine populations. In our preliminary results, for three analyzed genes we identified different SNPs between individuals origin from one population. It seems that proposed methods can be useful as a complementary research in the molecular diversity study based on the length of the DNA fragments (SSR). SNP analysis are still a quite new method in the field of molecular research, but till now it has been successfully used in the analysis of population genetic structure, the individual identification or the impact of non-synonymous mutations on the phenotypic variation in many species. There is a strong need of research development in this area, due to the amount and complexity of the factors affecting the polymorphism of the single nucleotides in the Scots pine genome.

Poster number : S5.18

The rare and private alleles/haplotypes as a measure of richness of gene pool in Scots pine (Pinus silvestris L.) populations in Poland

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Most of renevals in Polish forests are introduced in an artificial growing way by planting earlier cultivated material in forest nurseries. Investments in infrastructure of highly specialized nurseries are planned, what will meet the demand for high quality planting material.The aim of the project was to determine the size of the gene pool and genetic diversity (especially in terms of occurrence of rare alleles and haplotypes) of planting material, which has been grown in the traditional nursery (bare-root plant) and containerized nursery as seedlings with soil-covered root systems and comparing it to the genetic structure of trees, the seeds to renewal of artificial breeding have been collected from.Genetic structure of the maternal and progeny populations was determined by polymorphism analysis of microsatellite sequences of nuclear DNA (nSSR) using three loci SPAG 7.14, SPAC 11.6, SPAC 12.5 and chloroplast DNA (cpDNA) using three loci PCP36567, PCP71987, PCP87314. The experiment was set in the Forest Districts of Olsztynek and Oleszyce.The rare alleles/haplotypes have been listed in both populations of Olsztynek Forest District and Oleszyce Forest District, and in subpopulations: maternal generations, both progeny generations - from seed beds and from containers.Both populations of Scots pine which were analyzed in this study were characterized by a relatively large group of rare alleles for 4 loci nSSR and reached about 22%. In the context of 3 loci cpSSR population of Olsztynek Forest District has been characterized by a 42% of rare haplotypes, and population of Oleszyce Forest District had 23% of rare haplotypes.The rare alleles/haplotypes, both in nSSR and cpSSR, were most numerous in the planting material which have been produced in containerized nursery (for nuclear DNA respectively, 18% and 17% in subpopulations Olsztynek and subpopulations Oleszyce; for chloroplast DNA respectively 37% in subpopulation Olsztynek and 27% in subpopulation Oleszyce).Also private alleles and haplotypes have occurred most frequently in group of seedlings which were produced under controlled conditions in greenhouses.

Poster number : S5.19

Use of microsatellite markers for clonal identification in Norway spruce and silver fir seed orchards

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The Simple Sequence Repeats (SSR) method of DNA analyses were used for clonal identification in Norway spruce (Picea abies /L./ Karsten) and silver fir (Abies alba Mill.) seed orchards. Total genomic DNA was extracted by from young needles of sampled trees taken from Norway spruce and silver fir seed orchards using DNA Plant Mini Kit (QIAGEN). We analysed 164 Norway spruce trees from 21 different clones and 58 silver fir trees from 20 different clones. Norway spruce samples were screened by nine selected polymorphic nuclear microsatellite markers and for analyses of silver fir samples were used eight selected polymorphic microsatellite markers. PCRs were optimized for tested primers that have been scanned in publications (Scotti et al. 2000, Rungis et al. 2004, Melnikova et al. 2012, Hansen et al. 2005; Cremer et al. 2006). Fragment sizes were determined on capillary electrophoresis (Applied Biosystem 3500). The obtained data were analyzed using the statistical programs CERVUS and GenAlEx 6.501. Declared clone affiliation in Norway spruce seed orchard was confirmed in 94% of sampled trees and in 71 % of tested clones. Declared clone affiliation in silver fir seed orchard was confirmed in 76 % of sampled trees and in 50 % of tested clones. The identified genetic loci have been verified ashighlypolymorphicand could be further used for clonal identification of Norway spruce trees and silver fir trees.Acknowledgements:This work was supported by the project of the Ministry of Agriculture of the Czech Republic – Resolution RO0116 (10462/2016-MZE-17011) and project No. QJ1330240.

Poster number : S5.20

Mapping of genetic structure in selected populations of Picea abies (L.) Karsten in the Czech Republic

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The Norway spruce (Picea abies L. cv. Karsten) is the most economically important tree species in the Czech Republic used to be grown on more than 50% of forest land. To ensure superior reproduction material and maintain ecological stability of forests with high biodiversity, knowledge about genetic structure of natural spruce populations is needed. Simple sequence repeats (SSR) method was used for determination of genetic variability within and among twelve Norway spruce populations (SM01-SM12) collected mostly from gene reserves in different parts of the Czech Republic. Samples were screened by nine polymorphic nuclear microsatellite markers (Scotti et al. 2000; Melnikova et al. 2012; Rungis et al. 2004) and PCR products were analysed on capillary electrophoresis (Applied Biosystem 3500).Across all populations a higher genetic diversity (expressed as Shannon's information index; I = 2.12 ± 0.11) was observed with the highest value in the SM02 population (I = 2.3) from Orlic mountains in contrast to SM08 population (I = 1.9) from Bohemian–Moravian Highlands. In addition, relationships among Norway spruce populations based on genetic distance revealed the longest genetic distance between SM02 (Orlic mountains) and SM06 (Bohemian Forest) in comparison to the closest one between SM01 (Polabí) and SM05 (Central Bohemian Hills) populations both original hurst ecotypes of Norway spruce. Very low genetic divergence among all studied populations (FST = 0.008 – 0.031) was detected. In summary, our results indicate high genetic variability in tested populations which could be straightly utilized in forest management strategy. Acknowledgements: This work was supported by the project no. QJ1230334 and project of the Ministry of Agriculture of the Czech Republic – Resolution RO0116 (10462/2016-MZE).

Poster number : S5.21

Evaluating of genetic diversity of natural beech populations (Fagus sylvatica L.) in the Czech Republic by nuclear microsatellite markers

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Fagus sylvatica L. (beech) is economically the most important broadleaved tree in the Czech Republic, where are favourable conditions for its growth. Unfortunately, in the 19th century a strong decline of beech cultivation occurred as a consequence of artificial reforestation and silvicultural preferences for coniferous, especially Norway spruce. Recently, the area of the main coniferous, such as spruce, pine and larch is gradually decreasing in the Czech Republic while there is increasing proportion of silver fir and deciduous trees like beech. In order to reintroduce this species in larger extent, it is important to acquire more detailed knowledge about the dynamics of genetic diversity within and among beech populations. To provide insight into the genetic variation within and among 13 beech populations (BK01-BK13) growing mostly in gene reserves, protected landscape areas or National nature reservations in different parts of the Czech Republic we used simple sequence repeats (SSR) method for DNA analysis. Fourteen polymorphic nuclear microsatellite markers searched in the literature (Vornam et al. 2004; Asuka et al. 2004; Pastorelli et al. 2003; Pluess et al. 2013; Lefèvre et al. 2012) produced 3 - 19 alleles in 390 individuals. Calculations of genetic diversity parameters were performed using the statistical programs GenAlEx 6.5 (Peakall, Smouse 2006, 2012). The genetic diversity expressed as Shannon's information index values was the highest in the BK08 population from Loučná nad Desnou with value 1.747 whereas the lowest value 1.614 was in the BK11 population from Žákova hora, Cikháj. Observed heterozygosity of beech populations ranged from 0.613 to 0.702. Genetic distances between populations were calculated based on Nei's standard genetic distance (Nei 1972). The longest genetic distance (0.241) appeared between the BK03 (Hluboká) and BK12 (Frýdlant v Čechách) populations. The closest genetic distance (0.054) was between the BK04 (Buchlovice) and BK07 (Děčín) populations. Use of selected nuclear microsatellite loci proved a higher level of genetic diversity in studied beech populations. Acquired knowledge about the genetic characteristics of selected beech populations can contribute to reforestation management strategies of this species.Acknowledgements: This work was supported by the project of the Ministry of Agriculture of the Czech Republic – Resolution RO0116 (reference number 10462/2016-MZE-17011) and project no. QJ1230334).

Poster number : S5.22

Towards a Genome Diversity Atlas for tropical pine tree species grown in Southern Africa and South America

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Tropical pine species such as Pinus patula, Pinus tecunumanii, Pinus greggii and Pinus oocarpa form the basis of the softwood plantation industry in South Africa and several countries in South America. Natural populations of these species in Mexico and Central America are threatened and gene conservation has therefore become a high priority. Characterizing the amount and structure of genetic diversity in provenance trials, breeding populations and conservation parks established for tropical pines will aid in gene conservation, breeding and the development of genomic resources. Furthermore, it will allow species and hybrid discrimination and support the development of hybrids with tolerance to Fusarium circinatum (pitch canker), while retaining favourable traits such as fast growth and cold tolerance. We have analyzed 31 microsatellite DNA markers in 200 P. patula, 200 P. tecunumanii (95 high and 105 low elevation) and 150 putative F1 hybrid trees. To evaluate discrimination power, we simulated marker panels with loci exhibiting HO ≥ 0.7, PIC ≥ 0.7, different numbers of private alleles and contrasting allele frequency distribution. The results were used to prioritize 17 microsatellite markers for the development of a multiplex marker panel optimized for

species and hybrid discrimination. Bayesian based admixture analysis in STRUCTURE was then used to test the ability to assign individuals from the three reference sets to distinct genetic groups. Some provenances with low FST and high proportion of admixed individuals (e.g. San Jorenino, Guatemala) were subsequently removed to produce operational reference sets for each species. A secondary STRUCTURE analysis using the restricted reference sets allowed discriminating hybrids from pure species. The marker panel is being tested in an expanded group of tropical pine species to establish a non-redundant reference set for transcriptome-wide SNP marker discovery and the future development of a Genome Diversity Atlas for these species.

Poster number : S5.23

Genetic diversity and structure of Eastern peripheral Abies alba populations in Romania

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Due to the environmental changes or anthropogenic influences, the peripheral populations may be exposed to risks. In Romania, the silver fir populations located at the eastern margins of the species distribution range grows remarkably and has an adequate natural regeneration, but are under the pressure of the limiting factors - as relatively cold and dry climate - and requires special protection measures.In this study, we investigate patterns of genetic diversity across 19 silver fir (Abies alba Mill.) populations located in the Romanian Eastern Carpathians (REC), sampled by a gradient of ecological and climatic conditions, using 13 nuclear microsatellites. For comparison, other seven silver fir populations from Romanian Carpathians were also surveyed. We conducted data analysis at two levels: (i) all 26 populations and (ii) central versus ecological marginal populations (at regional level).The genetic diversity parameters, as expected heterozygosity (He) and the allelic richness indicated a relatively higher genetic diversity in all the populations, with no significant differences between the marginal vs. central populations. The AMOVA analysis of genetic differentiation indicated that the differentiation was relatively low; 95% of observed variation is due to within populations. Main groups of populations were distinguished, two in the eastern group being different as homogeneity: one group more homogenous (the Northern part of REC populations) and another one less homogeneous (the South-Eastern part of REC populations). The geographical and environmental drivers of the observed genetic structure were discussed and the concluding results indicate that the population from the eastern margins preserv valuable gene pool.

Poster number : S5.24

A closer look on genomic selection accuracy – what are we predicting?

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Several studies in conifer species and eucalypts have provided proofs of concept for genomic selection (GS) and showed its potential usefulness for selecting superior stock in tree breeding. GS is considered a breeding tool that allows for selecting genotypes at a seedling stage, or even from seed and somatic tissue and therefore reduces the time needed to complete a breeding cycle. However, the efficiency of GS appears dependent on shared pedigree between populations used for model training and the trees for which phenotypes or breeding values are predicted. GS accuracy degrades very quickly when models are validated in unrelated breeding stock.We investigated GS models in second generation spruce breeding populations with the aim to better understand the base of the observed model accuracy. In a black spruce genetic test repeated on two contrasting sites, tree height and

diameter growth as well as wood density and microfibril angle were recorded. A sample of 730 trees from 34 full-sib families was genotyped for about 5000 SNP markers or around 2.5 markers per cM.GS model accuracy – the correlation between true and genomic estimated breeding value – was generally high and values above 0.8 were estimated for all wood quality and growth traits. As expected, model accuracy dropped to zero when models that did not share any common parents were used for validation. Around 2100 SNPs were mapped to corresponding homologous genes on the syntenic and collinear white spruce linkage map, and allowed for distinct modelling of markers belonging to separate linkage groups. Small but non-significant differences in selection accuracy were found between models considering different linkage groups. However, overall accuracy was close to accuracy achieved with random marker subsamples of same size. We conclude that, at current marker density, GS model accuracy is mainly based on transmission of large haploblocks and relatedness in experimental layouts currently used in tree breeding.

Poster number : S5.25

Utilising genomics to assess adaptive potential in an important restoration species, Eucalyptus microcarpa: Comparing genomics of natural and revegetated stands

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The conservation and restoration of vegetation alone does not necessarily equate to the long-term sustainability of plant populations (Broadhurst 2013). To ensure evolutionary potential under climate change, ecological restoration relies on capturing high genetic diversity, especially adaptive diversity. As the traditional use of exclusively local material for restoration is challenged (Breed et al. 2013; Havens et al. 2015; Prober et al. 2015), understanding species' adaptation to climate will be critical for identifying appropriate seed sources to create robust restoration (Sgrò, Lowe, and Hoffmann 2011; Weeks et al. 2011; Hoffmann et al. 2015).Eucalypts are a dominant species across many landscapes in Australia and a key stone species in ecological restoration. Whilst studies are emerging (Bradbury, Smithson, and Krauss 2013; Dillon et al. 2014; Steane et al. 2014), knowledge of the genomics underlying climate adaptation in Eucalypts remains limited. Utilizing the power of genomics, we employ DArTseq, a reduced-representation genomics approach, to investigate adaptation and its implications for seed sourcing under climate change in Eucalyptus microcarpa, an important restoration species used extensively across agricultural south-eastern Australia. In particular, we investigated genomic adaptation using samples collected across climatic gradients of E. microcarpa and compare the genomics of natural stands to revegetated sites. Together this works aims to firstly, identify potential adaptive variation and associated environmental factors across the species' distribution, and secondly, assess how well current restoration captures genomic diversity, in particular adaptive variation. The presence of outlier loci and their association with climatic variables such as mean annual temperature, temperature in the wettest quarter and water stress, suggest climate adaptation in E. microcarpa. Greater differentiation between, and lower genomic diversity within, restoration sites compared to natural sites suggest that E. microcarpa restoration sites however, only partly capture landscape-wide genomic diversity patterns. Through this study we demonstrate the applicability of genomic adaptation studies to conservation under climate change. In particular, how adaptive genomic studies can provide deeper insight into the genomics of restoration as well as assist in defining appropriate seed sourcing under climate change.

Poster number : S5.26

Population genetics of Betula pendula Roth marginal populations in their Southern European limits

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During the last glacial age, in Southern Europe tree populations moved in the southernmost latitudes on the Italian Appenines and Southern Balkans and colonized lower altitudes as well. All major biomes in Europe contain Marginal/Peripheral (MaP) populations of forest tree species, whose persistance is threatened by climate change. Under these circumstances, tree populations are moving northwards and at higher elevations, but species ranges are limited by biotic and abiotic constrains, that can differently affect the isolation of the populations.One of the species facing this kind of threat is silver birch (Betula pendula Roth), whose distribution covers uniformly almost all Europe, but in the western and southern parts of the distribution range, like in the Iberian peninsula, Greece and Italy, its presence is patchy and confined at the higher altitudes only.The genetic pattern of silver birch in the main distribution area is well studied, whilst very little is known about the amount and organization of genetic variation in the southern marginal areas. Refugial areas, as those cited above, are theoretically expected to show higher genetic variability compared with surrounding recolonized regions.Herein, parameters of genetic diversity and structure (such as A, Ho, He, FIS FST) are presented for different MaP populations from Italy and Greece are. Based on these indexes, suggestions regarding the causes of differentiation among populations, and the partitioning of genetic variation within or between populations are presented. The analyses of the genetic structure of these populations point towards the existance of diverse gene pools exhibiting differential genetic variability. The influence of environmental complexity in the genetic differentiation observed is also addressed. The potential peculiarities of marginal birch populations compared to core population diversity are discussed.

Poster number : S5.27

Comparing genomic prediction models for the breeding program of the Eucalyptus robusta in Madagascar

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Eucalyptus robusta is one of the most socially important forest tree species in Madagascar. Plantations covered around 150 000 ha and 90% of Malagasy people are using woods or charcoal for the domestic energy. This species has been introduced in Madagascar in the end of the 19th century. The plantations established by smallholders are characterized by a low genetic diversity and a high level of inbreeding. There is a need to create a new breeding population to start a new improvement program and produce the future varieties able to meet the woods demand.A provenance trial including 1314 individuals from 19 provenances covering the natural range of the species in Australia has been

established in Madagascar in 1993. We explored the potential of this material as the basis of the new breeding population. To achieve this, 415 individuals already preselected on the basis of their phenotypes were further genotyped with 10 000 SNPs and 3 000 SNPs presenting no missing data were retained for investigating genotype-phenotype relationships and developing marker-based prediction models. Three statistical methods were tested, elastic-net (EN), genomic best linear unbiased predictor (GBLUP) and reproducing kernel Hilbert space regression (RKHS). Regression models could account for the population structure which was previously inferred by the STRUCTURE software. The individuals with highest genetic merit will enter the new breeding population. The results showed that the entire natural range of the species can be structured into three clusters including 2, 8, and 9 provenances respectively. Each individual within a provenance was assigned to a single cluster and this information was included in the prediction model. For height at 49 months we showed that there were differences between the three methods. Based on 100 cross-validations, the mean accuracies were 0.13 [sd=0.07], 0.27 [sd=0.05] and 0.28 [sd=0.05] for EN, GBLUP and RKHS methods respectively. In addition, the broad sense heritability estimated with RKHS was 0.10 [sd=0.05] and the narrow sense heritabilities estimated with EN and GBLUP were 0.06 [sd=0.08] and 0.08 [sd=0.05] respectively.

Poster number : S5.28

Tropical forest genetics: contribution to the planetary environmental crises

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Our planet is facing two major environmental crises, climate change and loss of biodiversity. Recognizing the urgency of the threats, Countries under the United Nations are promoting initiatives to slow down the degradation and move toward a more sustainable development. Tropical forests represent a vital answer to the environmental crises, as recognized by the Bonn Challenge (2011), the New York UN Climate Summit (2014), and the Paris COP21 (2015): they stock two-thirds of terrestrial biodiversity, 46% of the living carbon, and 23% of the annual CO2 emissions are generated by deforestation (Skutsch et al 2007, van der Werf et al 2009, estimates corrected for peat land fires). Slowing down deforestation is a very cost-effective mean to lower CO2 emissions, also supporting biodiversity maintenance, water cycle regulation and local socio-economic development. Acknowledging this, collective efforts of tropical Countries, world-level industrial groups and local ONGs add up today to an engagement of 2-300 Mha to be progressively reforested in the next 10-20 years.This planetary-scale effort represents the largest environment restoration bid ever undertaken by Man, an historical event not to be missed by forest biologists. If forest geneticists accept the challenge of forest restoration in the Tropics, several questions quickly emerge: Which tree species should be preferred? Which genetic resources? What are the challenges of producing tens of billions of plants? How to manage the necessary adaptation of genetic materials to site conditions? What could be the interest of even a minimal investment in research? Many of these questions are included in the new strategic plan of CIFOR. Among the ideas, the main one is to promote the emergence of regional R&D hubs to develop scientific and technical knowledge on indigenous species of interest for reforestation, for most of which the knowledge available is extremely limited today.The R&D hubs would develop a three-folded activity: 1. Generating scientific knowledge on species complex diversity, adaptation, growth, wood quality and multiplication, with studies from genetics and genomics to integrative biology; 2. Transferring actual knowledge to field operators and teaching facilities through training; 3. Supporting the establishment of networks of seed-gardens. Our AGAP group is interested to develop one of these hubs in French Guyana, and contribute to others in Africa (Madagascar) and South-East Asia (Borneo), investing in scientific research and development both in the field and in our labs. Through this poster, our group is kindly soliciting a dialogue with experts and interested partners that could enrich our vision.

Poster number : S5.29

Comparative analysis of gene dispersal based on SSR and SNP markers

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Single nucleotide polymorphisms (SNP) and microsatellites (SSR) were compared as genetic markers for parentage-based modelling of gene dispersal. Genotypic data based on 16 SSR and 118 SNP loci, and locations of adults and seedlings of Fagus sylvatica were used to infer seed and pollen dispersal patterns based on the seedling neighborhood model. For comparative analyses a basic version of the model was used, with the following parameters: seed immigration rate, pollen immigration rate, average seed dispersal distance and average pollen dispersal distance. Because both marker types are prone to genotyping errors, the model was upgraded to take into account genotyping errors in estimation procedures, with locus-specific error rates considered as estimable parameters.When genotyping errors were not taken into account in the model, the estimates of immigration rates were rather high. For SNPs, seed and pollen immigration rates reached 20% and 80%, respectively. Similarly, in the case of SSRs, seed and pollen immigration rates were found to be 10% and 82%. However, when typing errors were taken into account, the immigration rates decreased substantially with seed immigration being not significantly different from zero for both marker types. On the other hand, pollen immigration rate remained high (44% vs. 68% for SNPs and SSRs, respectively) and appeared significantly different between the two marker types. Seed dispersal distances were quite similarly estimated for SNP and SSR markers, regardless of whether or not genotyping errors were accounted for. Standard errors of the parameter estimates were comparable between SNPs and SSRs, and only slightly affected by the treatment of genotyping errors.Estimated mistyping error rates varied considerably among loci. In the case of SSRs, 7 out of 16 loci showed an error rate significantly greater than zero. Although genotyping errors are common for SSR markers, our analyses revealed that some SNP loci were also affected by genotyping errors. Although only 9 out of 118 SNPs exhibited significant error rates, such low-intensity scoring problems can accumulate rapidly with a large number of loci resulting in an upward bias in immigration rates.SNPs and SSRs provided fairly similar results and we conclude that they may be efficiently used as genetic markers for pollen and seed dispersal studies, but only when genotyping errors are accounted for in the model. However, such analyses for large datasets and/or more complex models based on SNP data may become computationally more demanding, calling up for a software upgraded for parallel computing.

Poster number : S5.30

Understanding eco-evolutionary responses to climate change using the genomics of local adaptation and genotype-by-environment interactions

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Population genomic patterns may carry signatures of local adaptation to environment. By uncovering the genomic basis of adaptation we may uncover a basis for predicting adaptive responses to environment and genotype-by-environment interactions (G×E). A central question is how populations differ in their responses to environmental change, the answer to which depends partly on patterns of genetic diversity. Here, I develop approaches to predict G×E using population genomics and I demonstrate how local adaptation contributes to eco- evolutionary dynamics of communities (e.g. forest trees) under climate change. First, I use published genomic data on >1,000 natural accessions of Arabidopsis thaliana and four common gardens across Europe to to predict the optimal genotype for a given environment and to predict genetic variation in reaction norms. I quantify spatial variation in genetic diversity of climate response and link these patterns to polygenic models of local adaptation. Second, I ask how differences in genetic diversity of locally adapted populations of competing species affects community response to climate change. I use numerical simulations to demonstrate that patterns of local adaptation and evolutionary response to climate change alter interspecific competition and community dynamics. Together, these results suggest that understanding the genomics of local

adaptation will enhance our ability to predict populational and community responses to climate change.

SESSION 6

Poster number : S6.1

A statistical model for estimation of apomixis rate of a tree species and its application to hickory (Carya cathayensis Sarg.), an apomictic species

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For random mating of a tree species in a natural population, a data set of molecular markers and a likelihood function were established when outcross, apomixis and selfing were considered, respectively, and EM (Expectation-Maximization) algorithm was used to estimate unknown parameters including rates of outcross (ω), apomixis (θ) and selfing (1-θ-ω). Significance of outcross and apomixis occurrence was tested and computer simulation was performed for testing the statistical model in terms of two sample sizes (200 and 400). It showed that the model developed could well estimate all the parameters, and the result would be better when the sample size was increased. Hickory (Carya cathayensis Sarg.) is an important non-timber species in Zhejiang Province and there has been no cultivar developed under this species. Studies conducted cytologically in recent years have shown that apomixis exists in this species (Zhang et al., 2012). With a parental population (40 individuals) from a natural population and its half-sib progeny (10 individuals each parent) resulting from open pollination, real data of 168 polymorphic loci resulting from RAPD (Random amplified polymorphic DNAs) and SRAP (Sequence-related amplified polymorphism) markers were analyzed with the model developed. It has been shown that ω, θ and 1-θ-ω could be estimated and the apomixis rate in hickory was around 32%, which indicated that apomixis existed at the DNA level in hickory.

Poster number : S6.2

An optimized MRE-seq strategy for the de novo assembly of the gene space of large and complex plant genomes

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Genome assembly remains a challenging issue for large and complex plant genomes predominantly due to their high amount of repetitive regions. Thus, DNA enrichment strategies facilitate the assembly by increasing the coverage and simultaneously reducing the complexity of the whole genome. Based on the fact that the gene space in plants is showing rather low methylation levels (hypomethylated) and that, in contrast, cytosine methylation is found predominantly in repetitive elements (e.g. transposable elements) [1], methylation-sensitive enzyme-based genome digests are emerging as a tool to enrich for gene related sequences [2].With the present study performed on Norway spruce (Picea abies (L.) H.Karst.) and rice (Oryza sativa L.) we demonstrate that with an optimized methyl filtration protocol with subsequent next generation sequencing, a variant of MRE-seq, the hypomethylome and thus the gene space of a plant can be easily accessed by the use of methylation-sensitive restriction enzymes [3]. The NGS data was analyzed using different approaches illustrating the comparability of de novo assembly and reference based mapping. Sequenced data sets of hypomethylated fragments show a substantial depletion of repetitive regions together with enrichment for transcribed regions. Furthermore, a clear increase of sequencing coverage in active genomic regions can be observed. As the method targets genomic sequence not only within but also around genes, many important components are also represented including introns and potentially regulatory

regions of promoters.Our method provides an easy tool for killing two birds with one stone: (1) the reduced representation library enriched for gene space can serve as cost-effective tool for analyzing a plant's gene space depleted of repetitive elements comprising over 50–80% of the genome; (2) with this representation of the hypomethylome, an easy comparative analysis of epigenetic variation among genotypes or tissues can be performed at an affordable price, even in a larger set of samples.

Poster number : S6.3

Association studies and marker identification in Norway spruce facilitated via a novel SNP array

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Association studies are a powerful approach to identify marker-trait associations both in model and non-model species. Relevant markers can be detected by identifying statistical associations between DNA sequence variants in single nucleotide polymorphism (SNPs) and the variation of a target trait in a population. Besides providing insights into the genetic basis of naturally occurring complex phenotypic variations, this technology facilitates the analysis of large populations and of several alleles at once. Association mapping has so far been performed successfully in coniferous forest trees for several phenotypic traits, including wood properties, growth and wood chemistry, serotiny, carbon isotope discrimination, cold hardiness and bud set timing, metabolite concentrations and disease resistance. For Norway spruce (Picea abies L.), only studies focusing on chlorophyll a fluorescence, frost resistance, height, diameter, bud burst, and bud set have been published. While for model species common sets of SNPs are available, those studies in Norway spruce have been based on different sets of SNPs.We present a custom Illumina Infinium iSelect HD BeadChip for Norway spruce integrating 3,257 SNPs chosen from different resequencing and genotyping projects. The developed array includes 3,116 SNPs located within the gene space (exons, introns, and 1,000 bp up- and downstream), allowing associations with specific genes and to investigate potential regulatory effects of the marker with the associated gene. Besides the possible association with genes, a total of 1,240 neutral SNPs allow the analysis of potential genetic structures within any analysed population – an important aspect of each association study. The presented SNP array has been successfully applied in different studies aimed at analysing marker-trait associations including metabolite content and resistance associations, stress tolerance, developmental and growth behaviour, and genetic structure among populations of N. spruce. Over 1,290 analysed samples, the SNP array showed an average call rate of 88 % ranging from 32 % to 96 % per sample providing a sufficient base for association mapping tests for each experiment.Acknowledgments: This study was kindly supported by the Austrian Research Promotion Agency (#834209), Forstbetrieb Mayr-Melnhof, Kooperationsplattform Forst Holz Papier, Lieco, and Österreichische Bundesforste.

Poster number : S6.4

DNA extraction from oak heartwood: motivation and challenges

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The wood of forest trees offers a unique opportunity for centuries-spanning longitudinal studies of the relationship between plant growth and environmental factors. The environmentally modulated year-to-year variations in macroscopic features of growth rings in wood are the basis for dendroclimatology and dendroecology studies that aim to reconstruct past climate conditions and to predict the effects of climate change on tree growth. However, wood also is a molecular archive of past growth conditions: even after the terminal sapwood to heartwood transition, it remains a viable source of archival DNA. This DNA provides an opportunity to expand dendroclimatology and dendroecology to include epigenomic features that may convey additional and/or more specific information on past environmental conditions compared to the macroscopic features used so far. Specifically, genome-wide DNA methylation profiling followed by epigenomic association studies could identify genomic regions (including genes) where DNA methylation changes systematically relate to changes in a particular environmental factor. However, the purification of DNA from heartwood is challenging. Heartwood DNA extracts often contain large amounts of polyphenols that inhibit downstream

enzymatic reactions. And while polyphenols can preserve DNA from degradation via the inhibition on nucleases, equally, in the presence of metal ions, they can contribute to DNA degradation. For these reasons, effective protocols to obtain polyphenol-free DNA extracts are desirable. We are testing ascorbic acid, β-mercaptoethanol and polyvinylpyrrolidone (PVP) for their ability to reduce content and oxidation of polyphenols during oak heartwood DNA extraction. Post extraction, we are exploring means to further purify the DNA, including DNA dialysis and protein-polyphenol nanoparticle formation reported for albumins and mucins. We thus aim to develop a DNA purification protocol that will contribute to the unlocking of the epigenome archive preserved in the wood of forest trees.

Poster number : S6.5

On the demarcation of forest genetic monitoring regions

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Genetic monitoring has proven to be an essential tool for genetic conservation across biomes. Forest genetic monitoring is the quantification of temporal changes in population genetic variation and structure, allowing the assessment of the dynamics of transition from the present to the future genetic status of a forest stand. The major genetic monitoring characteristic is the temporal evaluation of the population genetic status. Several theoretical considerations and practical implementation aspects are associated with forest genetic monitoring. In a cascade of relevant research and implementation processes, the identification of forest genetic monitoring regions is a leading priority. Herein we describe the formalization of a combined data-driven and expert-based approach that leads to the demarcation of forest genetic monitoring regions. This approach is founded upon the natural distribution of the species, the available environmental zonation/stratification information, the existing genetic data, as well as local expert knowledge. Within the LIFE+ project LIFEGENMON, this approach is being tested; in particular, in a NW to SE transect spanning from Bavaria to Greece for seven priority species with contrasting biology, ecology and distributional properties: the Abies alba - A. borisii regis complex, Fagus sylvatica, Fraxinus excelsior, Pinus nigra, Populus nigra, Prunus avium, and the Quercus robur - Q. petraea complex. Results regarding both the formalization of the approach, and its implementation at the species level within the geographical context considered, are presented and discussed.

Poster number : S6.6

breedR: Statistical methods for forest genetic resources analysts

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Phenotypic records have gained in depth and complexity in the field of forest tree genetics and genomics, thanks to cumulated historical data and the raising of new high-throughput screening techniques.This leads to highly structured data such as multivariate, longitudinal, genomic or multi site. Their interplay with complex effects can quickly become overwhelming and difficult to handle.The

breedR (Muñoz and Sanchez 2016) package provides frequentist and Bayesian statistical tools to build and fit a large variety of models, while taking care of most of the technical details. It is able to handle effects that are useful in forest trials such as spatial autocorrelation, competition, permanent environments, genotype-by-environment interaction, and environmentally-based plasticity, to cite just a few.breedR implements all models as a Linear Mixed Model, builds all the required incidence and structure matrices and relies on the BLUPF90 (Misztal 1999) suite of FORTRAN programs, providing reliable REML estimation or Gibbs sampling.In addition, it retrieves the results into convenient R-objects for subsequent inspection, diagnosis or plotting. BreedR is undergoing active development as part of the Trees4Future and ProCoGen projects under a Free and Open Source license (GPL-3), and backed by a rapidly growing community. We illustrate some of its capabilities using a case study on spatial analyses.

Poster number : S6.7

De novo transcriptome assembly and molecular marker detection in Spanish fir (Abies pinsapo)

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The Spanish fir (A. pinsapo) can be found only in five cores in the world. In Spain, these populations are located in Málaga and Cádiz (Linares and Carreira 2006). They are distributed between 1,000 and 1,800 metres in altitude, always in shady and north-facing slopes where they can receive abundant orographic rainfall. In northern Morocco, there are two varieties of the A. pinsapo: variety tazaotana is located in Mount Tazaout, at 1,400 – 1,700 meters in altitude, and variety marocana spreads out in the Chefchaouen Mountains, at an altitude range of 1,400 – 2,100 metres (Liu 1971). Increases in mean annual temperatures and inter-annual precipitation variability in areas were Spanish firs are located appear to be responsible - at least in part – of the symptoms of growth decline observed since the 1950s (Linares et al. 2010). Also, in the last decade there has been a significant drop in its basal limit distribution, which reaches 20% of dead basal area (Linares and Carreira 2006). This sensitivity to drought appears to have a special impact on youthful trees since it hampers their ability to reach maturity successfully in most of the cases (Génova Fuster 2008).To protect the Spanish fir a better understanding of its genome is necessary. Transcriptome analysis based on next-generation sequencing (NGS) is an effective tool for generating genomic resources and identifying polymorphic molecular markers for non-model organisms. Because of that, an Illumina de novo transcriptome sequencing has been performed in order to discover new insights into the genetics of this species. A 24 GB transcriptome dataset was obtained in total. In the results of the assembly, 97,768 unigenes were detected and were compared with the DroughtDB (Alter et al. 2014), in order to identify characterized genes involved in drought stress response. Several genes involved in this response has been identified, such as AP2/ERF, DREB, MYB, Heat-Shock or Dehydrogenase genes, all of them involved in drought-stress tolerance. These transcripts constitute potential candidate genes as a starting point in further studies related with abiotic stresses responses in A. pinsapo. On the other hand, the availability of this high-throughput sequencing data has led us to the discovery of specific SNPs (100,949) and SSRs (5,663). Transcriptome data obtained with this technology will allow us to develop a collection of molecular markers which will provide detailed insights into the genetics of Spanish fir populations.

Poster number : S6.8

An RNAseq approach to decipher the molecular mechanisms involved in the biosynthesis of the aromatic compounds in European white oaks wood.

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Aromatic compounds found in the wine mostly arise during the contact with oak wood barrels. Whisky-lactone which gives a coconut and a woody taste is the most important aromatic compound. Sessile and pedunculate oaks (Quercus petraea and robur) are widely used for wine maturation but these two species are characterized by a contrasted pattern for whisky-lactone. Identification of the genes involved in the biosynthesis of these compounds is of great importance to provide both (i) Gene based diagnostic markers for species identification and (ii) homogenous batches of barrels. However, the molecular mechanisms involved in the biosynthesis of the aromatic compounds remain in their infancy in forest trees. To fill this gap, we implemented an experimental design on 20 sessile and pedunculate oaks. First, wood cores were sampled on each individual. The transition zone between heartwood and sapwood was immediately harvested and stored at -80°C until RNA extraction. The remaining wood was grinded and aromatic compounds quantified using GC/MS or LC-HRMS. This chemical approach confirmed the result previously obtained by [1] and [2] namely a higher whisky-lactone and QTT content in sessile oak and a higher Glu-BA content in pedunculate oak. Second, three sessile and pedunculate oak samples were selected according to the chemical data. Total RNA were extracted from the transition zone and its gene expression was investigated by RNAseq. We found that 469 genes were differentially regulated between the two species using the Deseq and the EdgeR packages. Among these genes, 100 and 84 were specifically expressed in the transition zone of sessile and pedunculate oaks respectively. A functional annotation was performed highlighting molecular functions potentially involved in the biosynthesis of the aromatic compounds.

Poster number : S6.9

Nucleotide diversity of Populus nigra transcriptomes

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Lignocellulosic biomass from cultivated poplars is a renewable resource of interest for the production of bio-energy. To improve this complex trait, it is essential to better understand the genetic factors that affect its variation. Within this context, to explore the components of genetic variability within a parental species of commercial hybrid poplars, we have initiated an integrative approach combining genomic, transcriptomic and phenotypic information in a large collection of individuals sampled from natural populations and raised in a clonal field trial.Here, we report the analysis of polymorphism within transcriptomic sequences produced by RNA-seq. RNA was extracted from young differentiating xylem and cambium collected on 24 3-years old trees corresponding to 2 replicates of 12 genotypes from 6 natural populations representative of the geographical distribution of the species in Western Europe. After RNA quantification, the RNA from xylem and cambium from the same tree were pooled and subjected to high-throughput sequencing on an Illumina HiSeq 2000 using 100 bp paired-end indexing runs. All the samples were sequenced on one Illumina flow-cell, yielding around 18 million of paired-end reads per sample.The sequences were first trimmed (quality and length) and aligned to the Populus trichocarpa v3 assembly using BWA-MEM. Around 99.7% of the reads mapped against the reference genome and 93.3% properly mapped despite the fact that the genome is from a different species. Then, we performed a mapping filtering for duplicated sequence reads after alignment to the genome with Picard tools to mitigate biases introduced by data generation steps such as PCR

amplification. We also performed local realignment around indels and recalibrated the base quality scores with GATK. The variant discovery process was based on several variant callers (GATK, VarScan, FreeBayes) with several filtering parameters (VCF tools). We compared the genotype calls to those previously obtained with a SNP array (Faivre-Rampant et al, 2016) in order to choose the optimal variant caller and the SNP filtering parameters (depth, quality).The usefulness and relevance of the resulting SNPs for population genomic studies was further assessed by analyzing: (i) genome-wide patterns of nucleotide diversity, (ii) population structure, and (iii) linkage disequilibrium. The SNPs have also been annotated in order to estimate Ka/Ks ratio, with a particular focus on candidate genes involved in the control of lignocellulosic biomass production and quality.This promising approach is currently being extended to 240 genotypes in order to validate the genomics patterns identified in the present study.

Poster number : S6.10

Molecular characterization of transgenic maritime pine somatic plants overexpressing a cytosolic glutamine synthetase gene (PsGS1a) involved in nitrogen assimilation

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Nitrogen (N) is a nutrient usually available at extremely low levels in forest ecosystems. Thus, efficient mechanisms are required for the assimilation, storage, mobilization and recycling of inorganic and organic forms. Ammonium is the predominant N source in forest soil, and it is well documented that conifers, unlike herbaceous plants, have a preference for ammonium over nitrate as an inorganic N source. In conifer, the cytosolic glutamine synthetase GS1 is involved in ammonium assimilation and amino acid biosynthesis in both photosynthetic (GS1a holoenzyme) and non-photosynthetic cells (GS1b). Different approaches have shown that GS is a key component of plant N use efficiency, supporting a role of GS in plant development, growth and biomass production. A pine GS1a gene was constitutively overexpressed under the control of CaMV35S (P35S) or maize ubiquitin (Pubi) promoters in maritime pine following Agrobacterium-mediated genetic transformation of embryogenic tissue. Plants were regenerated from cryopreserved transgenic lines and appropriate non-transgenic (NT) controls through somatic embryogenesis. PCR analyses confirmed the transgenic status of somatic plants with a few T-DNA copies (1-3) integrated into the genome. Two-years-old plants from different lines were sampled for estimating GS1a transcript content, total protein content, GS1a enzymatic activity and also for GS1a protein detection. Results are highly variable between lines and even between plants from the same clone suggesting pleiotropic and/or (epi)genetic regulation effects. GS1a transcript detection was similar to NT controls. Increased total protein content and GS1a activity were observed in some lines and plants for both constructs. Two Pubi-or P35S-GS1a plants with high GS1a activity were selected for further transcriptomic profiling (9K PINARRAY2). These plants were confirmed to be upregulated for GS1a (RT-qPCR) and differentially expressed 527 and 685 genes compared to NT controls. Gene ontology terms could be assigned to 301 and 360 unigenes, respectively. A good concordance in functional categories was observed between both constructs with high preponderance of the "protein" category but also "photosynthesis" and "amino acid metabolism". More stringent selection criteria highlighted 12 commonly deregulated genes involved in stress resistance and growth. Strikingly, GS1a overexpression resulted in slightly improved plant growth behaviour in some lines but only during the first season at the GMO glasshouse containment.This research received funding from the European Community's FP7/2007-2013 programme (289841-PROCOGEN), The French National Research Agency and the Spanish Ministry of Science and Innovation (SUSTAINPINE: ANR-09-KBBE-007, BIO2012-33797). This work also benefited from equipments of the XYLOBIOTECH technical facility (XYLOFOREST platform, ANR-10-EQPX-16).

Poster number : S6.11

ELIXIR Portugal – integrating data in the woody plant domain

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ELIXIR Portugal is a consortium of Portuguese research institutions (INESC-ID, iBET, IGC, ITQB-UNL) which is part of the European Research Infrastructure for Life-Science Information (ELIXIR, http://www.elixir-europe.org/) and of the national biological information network BioData.pt (http://www.biodata.pt). Like ELIXIR itself, the Portuguese node is organized as a decentralized network of specialized centers, under a common computing infrastructure, and with shared training and industry/entrepreneurship programmes with a European dimension.Woody plants are a major natural resource in Europe, with a huge ecological and economical impact, supporting millions of jobs across diverse industries (e.g. wine, fruit, olive oil, coffee, paper, timber and cork) and strongly contributing to the European GDP. In Portugal, woody plants represent 10% of the GDP, and are a central research domain in both academia and industry.Massive sequencing and genotyping of woody plants (and their pathogens and pests) is generating large quantities of molecular data. Phenotypic data collected for each plant genotype, often in multiple locations and field conditions, has also been generated within different breeding programmes. Moreover, novel approaches are being implemented to identify and collect large sets of quantitative phenotypes and to explain the genetic basis of important traits.Elixir Portugal aims to secure and deliver the core data resources with a major focus on woody plant research, provide tools and services to drive data access and exploitation, develop and maintain controlled vocabularies and standardized APIs for data interoperability, reuse and integration, and provide training programmes in this domain.By focusing on genotype-phenotype analysis based on the widest available public datasets, enabling more powerful association analysis and opening the way to understanding of function, candidate gene prioritisation, and improved plant breeding, this initiative will contribute to build a framework that is of added-value to the woody plant user communities including industry and academia. Acknowledgements:FCT is acknowledged for financial support through project EXCL/EEI-ESS/0257/2012, and EU for support through H2020 project ELIXIR-EXCELERATE, grant agreement 676559.

Poster number : S6.12

SNP discovery and genotyping in Fagus sylvatica: comparative analysis of RAD and GBS methods.

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European beech (Fagus sylvatica L.) is one of the most important broadleaf tree species in Europe, which might be subjected to negative consequences of climate changes. However, relatively few studies attempted to identify gene loci exhibiting signatures of selection. We applied restriction site associated DNA sequencing (RAD) and 'genotyping-by-sequencing' (GBS) methods to a sample of 91 individuals of Fagus sylvatica, collected across a broad range of the species distribution. RAD libraries were prepared based on the PstI restriction digest, while GBS libraries employed ApeKI restriction digest. Resulting sequences were filtered for quality and coverage, and for each marker type two datasets were prepared: based on weak and strong data filtering protocols, using STACKS. Only loci present among >80% individuals were retained for further analyses, which resulted in 5086 - 6124 (GBS) and 6007 - 7857 (RAD) SNP loci.In order to identify loci that contribute to population differentiation, all individuals were ranked according to the four gradients. Three gradients were related to geographic locations of populations: longitude (WE), latitude (NS), composite climate score (PC1; the first component of PCA for 19 Bioclim variables, which explained 42% of variation), and one gradient was based on bud-burst phenology scores (Phen). For each gradient two subpopulations were established consisting of individuals of lower and upper quartile of a rank (20-24 individuals per subpopulation). Loci exhibiting signatures of selection were identified using FST-outlier approach implemented in Arlequin 3.5.In general, intensity of data filtering had a minor effect on RAD data; however, it largely reduced the number of loci in GBS, which might be related to the differences in restriction enzymes used and the protocol differences of the library preparation. Depending on the

intensity of data filtering (weak or strong) we identified 65 or 41 (RAD) and 157 or 46 (GBS) outlier loci, respectively. However, 7 loci were identified as significant FST outliers in two gradients. Comparable numbers of outlier loci were identified for each gradient, with the lowest numbers detected for longitudinal gradient.Alignment of nucleotide sequence reads containing the outlier loci with beech transcriptome indicated positive match for 20 (49%) RAD and 37 (80%) GBS loci (based on strongly filtered dataset). Considering the number of detected SNPs and outlier loci, as well as the costs of analyses, GBS markers seem to me more efficient for landscape genomic studies of beech, as compared to RAD markers.

Poster number : S6.13

Forest genetic monitoring: an overview of concepts and definitions

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Protecting and securing forest ecosystems with their habitat variability and all their functions is of highest priority. Therefore, the long-term adaptability of forest ecosystems to a changing environment must be secured, e.g. through sustainable forest management. High adaptability is based on biological variation starting at the gene level. Thus the ultimate goal of the Convention on Biological Diversity (CBD) to halt the ongoing erosion of biological variation is of utmost importance for forest ecosystem functioning. Monitoring of biological diversity over time is needed to detect changes that threaten these biological resources. Genetic variation, as the basis of biological diversity, needs special attention for its preservation, supported also by monitoring. We show the development of forest genetic monitoring (FGM) attempts in comparison to other biodiversity monitoring concepts. FGM enables the early detection of potentially harmful changes of forest adaptability before these appear at higher biodiversity levels, and can improve the sustainability of applied forest management practices and direct further research. Theoretical genetic monitoring concepts developed up to now need to be evaluated before being implemented on a national and international scale. This contribution provides an overview of FGM concepts and definitions, discusses their advantages and disadvantages, and provides a flow chart of the steps needed for the optimization and implementation of a forest genetic monitoring concept. FGM is an important module of biodiversity monitoring and we define an effective FGM scheme as consisting of an assessment of a forest population's capacity to survive, reproduce and persist under rapid environmental changes on a long-term scale.

Poster number : S6.14

Detection of WUE positional candidate genes by combining QTL maps and BAC sequencing with the annotated oak genome sequence

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Water use efficiency, estimated using carbon isotope composition (δ13C), represents the balance between carbon acquisition and water loss. It is an important trait affecting tree growth and economical use of water. Mapping of quantitative trait loci related to δ13C in a Quercus robur full-sib family revealed one major QTL stable across 3 years of measurement. This QTL is located on linkage group (LG) #11 of the male and female parental genotypes of this progeny (Brendel et al, 2008, Trees Genet Genomes 4: 263). The phenotypic variance explained by the QTL across different years varies between 21% and 31% on the male and between 3% and 5% on the female LG, respectively. More recently, EST-SSR and SNP markers were added to the maps (Bodénès et al, 2012, BMC Plant Biol 12:153, Bodénès et al, 2016, DNA Res In press). On the composite linkage map, the QTL interval was found to be flanked by one SSR marker and one SNP marker. This interval spans 1.8 cM and

comprises 3 other SNP markers. These markers were used to screen 2 BAC libraries constructed for the female parent (selected as a reference genotype for WGS) and arranged in 3D pools to identify BAC inserts corresponding to the genome region covered by the QTL confidence interval (Faivre Rampant et al, 2011, BMC Plant Biol 12:153). Five positive BAC clones were selected for insert sequencing. Both markers and BAC insert-sequences were aligned on the Q. robur genome assembly (Plomion et al, 2015, Mol Ecol Res 16: 254). Two scaffolds were identified and annotated using Eugene to integrate ab initio and similarity gene finding software (Sallet et al, 2014, Bioinformatics 30: 2659), and FunAnnotPipe an in-house pipeline mainly based on InterproScan to search for patterns/motifs and Blast based comparative genomics. Annotation identified 30 gene models including 17 known protein-coding genes, providing a first list of positional candidate genes to be further validated by functional genomics and association study.This research was supported by the ANR project H2Oak, ANR-14-CE02-0013

Poster number : S6.15

Genomic selection modelling of growth and wood properties in a Eucalyptus grandis x E. urophylla backcross population

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The development of genomic resources, like the sequencing of the Eucalyptus genome and the development of genome-wide genotyping tools, has made the concept of genomic selection (GS) more accessible in Eucalyptus species. GS promises to improve tree selection efficiencies and shorten the breeding cycle, while providing accuracies comparable to those derived from conventional phenotypic selections based on traditional analyses (BLUP). As a step towards this, GS models were developed for a Eucalyptus grandis x E. urophylla (GU) backcross population (seedlings and cloned siblings of various ages) using marker data derived from the EucHIP60K.Br SNP chip for key traits critical to the pulp and paper industry, including dissolving pulp yield, total lignin content and basic wood density. After marker filtering based on call rate (>0.9) and minor allele frequency (<0.05), a data set comprising 16,210 SNP markers analysed in 875 F2 backcross progeny was produced. Three different GS modelling analyses were applied (GBLUP, Bayes C and Bayes Cπ) using the Golden Helix software (v8.4.2). Validation of the GS models was performed using a 10-fold cross validation process. In general, the Bayes Cπ models showed the highest accuracies, with 0.91 for total lignin content, 0.86 for screened pulp yield and 0.73 for density. The RMSE values were very low (<1.43) and the heritability values for the traits ranged from 0.42 to 0.61. The allelic substitution effect (ASE) graphs showed similar patterns for all three models for most traits confirming that the different models are identifying the same SNP markers as having significant effects on the trait. These results are promising for the application of genomic selection in GU clones for various traits of interest. It should be noted that these GS models were developed with a very small effective population size. The linkage disequilibrium is very high in such a closely related population, resulting in very high accuracies of the models, but likely with low transferability to unrelated pedigrees. The next step in the testing of these models will involve the application of the developed GS models in a related set of GU backcross trees and expansion of the training population to include a more representative set of GU genotypes.

Poster number : S6.16

Mitochondrial variants in Pinus sylvestris and the P. mugo complex

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Whole-genome-shotgun (WGS) sequencing was employed for the discovery of new mitochondrial variants that may provide further insight into the history of P. sylvestris (L.) and the closely related P. mugo complex throughout Europe. DNA extractions were performed on bulked megagametophyte tissue from six mother trees from disparate locations across Europe and 100bp paired-end sequencing

was performed via the Illumina HiSeq platform. Using a similarity-based approach, approximately 1Mbp of putative mitochondrial sequence was recovered by comparison of our de novo assembled contigs with published sequences. In total, 31 novel SNP loci were identified following read mapping of shotgun sequenced samples. Primers targeting these loci were designed and Sanger resequencing was performed on a limited trial set of 28 individuals from across Europe. The geographic distribution of variants was consistent with results from previous studies, however, resolution was significantly increased by incorporation of the novel variants.

Poster number : S6.17

Genotyping and analysis of genetic diversity of trees "plus" Cork oak candidates (Quercus suber L.) of the Mamora forest in Morocco using microsatellite loci

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Mamora is a particular cork oak forest in Morocco by its production of acorns sweet and area one-piece plain. She knows a regressive dynamic accompanied by a degradation of the structures of the stands due to anthropogenic factors and mainly to lack of regeneration. Forty-seven systematically characterized candidate plus trees of Quercus suber L. from 5 cantons were analyzed by microsatellites markers (SSR). These genotypes considered best suited to natural selection were used to clonal propagation.The aim of this study is to evaluate genetic diversity and the genetic relationships among them and to establish DNA-fingerprinting for genotyping of tree.Thirty-nine alleles were generated with a mean of 5,57 alleles per locus and 8 and 9 for the two loci ssrQpZAG36 and MSQ4 respectively.The diversity analysis revealed a significant polymorphism (94%) and allelic richness with high observed heterozygosity in the different cantons, confirmed by the low and negative value of the fixation index (Fis= -0,319). A strong linkage between different populations with gene flow equal to 10.23 revealing the low inter- population variability. This result is supported by the low genetic differentiation Gst and Gst'' (0.022; 0,058) and a low variation between populations (Fst = 0.052) only 16 % revealed by AMOVA.Genetic typing of the entire collection of “plus” trees showed that there are 47 different genotypes (MQS11 to MQS57). Sixteen genotypes present specific markers facilitating their detection and traceability in case of multiplication. Eight unique alleles were identified and the specificity of these is distributed between the 5 cantons.This high genetic variability detected intra-populations of these trees and their genotyping can be of great use in breeding and conservation programs. It could be used to select genotypes of interest for the multiplication of seedlings for reforestation.

Poster number : S6.18

XYLOFOREST - a research infrastructure for the forest-based bioeconomy

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XYLOFOREST is a national research and innovation infrastructure for the forestry sector focusing on sustainable biomass production and wood utilization, from molecular level to final product level. Through multidisciplinary approaches combining forest ecology, tree genetics, silviculture, wood sciences and timber engineering, it links innovations and production processes along the forest value chain from the production of improved reproductive material and adaptation of forest systems to climate change to advanced timber engineering and industrial use of wood in biorefinery systems.The scientific objectives of XYLOFOREST are: (i) to experiment innovative and intensive wood production systems and assess their environmental balance and durability; (ii) to develop methods and tools for deployment of improved tree species for wood and biomass production; (iii) to increase understanding of molecular mechanisms for wood formation and wood chemistry through high throughput

measurements of phenotypic characteristics; and (iv) to improve technological processes for enhanced and sustainable use of wood in structures, composites, energy and chemical products.XYLOFOREST is structured in 6 technological facilities which are located in Aquitaine Region (South-West of France) with satellite sites in 4 other French regions. It offers access to high level equipments that are not available at this scale in Europe, and operates as an open research and innovation platform opened to scientists and public–private partnerships. Dissemination to stakeholders and exploitation of results are essential aspects of the infrastructure, as well as transfer of knowledge to students and use of facilities to support training activities.

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Session 6	Nathaniel Street, Greg Dutkowski, John Mackay, Jean-Marc Aury, Ross Whetten
GenomicSelection Workshop	Léopoldo Sanchez, Catherine Bastien

Other people who helped make the conference possible:

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