Meta-analysis of within-population genetic diversity across the Mediterranean Basin

Cyrille Conord, Bruno Fady

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Introduction

Diversity is one of the key elements needed to preserve the adaptive potential and capacity of organisms to adapt to new environmental conditions. There is therefore growing concern over erosion of all forms of biological diversity under increased human impacts and global change. Moreover, the complexity of ecosystems is tremendous. We are only now beginning to describe the structure of biodiversity and are still largely ignorant of the mechanisms underlying its evolution. In its broadest sense, biodiversity embraces ecosystem, species and genetic diversity. Within this hierarchy, DNA variation plays a pivotal role since it underlies the response of organisms and species to environmental change.

In the past decade genomics has became a sophisticated tool to decipher diversity at the most refined scales (genes and nucleotides).

There is a growing interest in exploring the functioning of ecosystems and their adaptation to changing environmental conditions not just in terms of their species diversity but, by starting with DNA molecules, studying organisms in the context of ecosystems. Genomics provides tools and information that allow understanding the mechanistic basis of these complex relationships among different organisms.

The aim of this international conference is to present new scientific findings in the area of ecosystem genomics, which addresses the structure and evolution of gene diversity at the population and community level. The conference will focus on the function and diversity of genes of adaptive significance in the context of climate change. Adaptation of forest ecosystems will be analysed from an evolutionary perspective and illustrated by examples on trees and their associated species.

The conference is a joint event co-organized by the research partners in the Network of Excellence EVOLTREE (www.evoltree.eu), funded by the EC 6th Framework Programme for Research and Technological Development. The event is open to the wide scientific community and has in its programme a dedicated session for policy-makers and forest practitioners, where the implications of research findings for the formulation of relevant policies and implementation of sustainable forest management will be discussed.

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Opening plenary session

PERSPECTIVES OF THE EUROPEAN COMMISSION’S ENVIRONMENT (INCLUDING CLIMATE) RESEARCH PROGRAMME

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Within the Specific Cooperation Programme of the European Commission’s 7th RTD Framework Programme (FP7; 2007–2013) the multi-annual Environment (including Climate) Theme supports transdisciplinary multipartner research projects on a wide variety of large research areas covering climate change, pollution and risks, the sustainable management of natural resources, the development of environmental technologies, and the development and application of earth observation and assessment tools to increase sustainability. Overall, in addition to increasing scientific knowledge, this research intends to underpin EU policy-making and create innovative problem-solving approaches for stakeholders at European level and beyond. Current EU-supported research efforts and the perspectives of forthcoming research within the second half of FP7 on the mitigation of climate change and environmental impacts and on the understanding of the role of biodiversity in forest ecosystem functioning and the resulting provision of goods and services in times of climate change will be highlighted. Also the perspectives of the application of modern genomic approaches to better understand the functioning of forest ecosystems at global level will be addressed.

Keywords: EU cooperative research, forest biodiversity, forest ecosystem genomics.

GLOBAL CHANGE AND FOREST GENETICS: ISSUES IN FUTURE FUNCTIONING OF POPULATIONS AND ECOSYSTEMS

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Future environmental conditions that determine forest functioning and structure are predicted to be significantly different from those of the past and the present, in some cases uniquely so. Consider the plant species that may be responding to both warming climate and increasing carbon dioxide, while the animals associated with those plant species respond only to the climate changes. Consider the insect pollinators that respond to changing late summer warmth while their target flowers continue to respond only to unchanging night length, seeding and senescing on normal schedules. Yet, despite these potential divergences between future environmental conditions and organism responses, the past responses of plants and forests can constrain estimates of requirements for population and ecosystem vigour in the future. For example, tree species migrated during the Holocene (the past 10,000-15,000 years) at approximately one fifth to one tenth the rates of projected future climate changes. Such measurements of prehistoric migrations provide initial estimates, which must be modified by conditions on modern temperate-zone landscapes. There, agricultural land uses will further reduce the migration rates of species with wind-transported seeds but can enhance migration rates of species with bird-transported seeds. Measures of ecosystem or biome responses to slowly changing Holocene climate reveal long time periods of biome composition unlike any on our modern landscape. The much more rapid climate changes expected in this century and beyond suggest even more unique ecosystems may become common. Attempts to model these responses suggest a transient depauperization of ecosystem composition as regional species extirpations outnumber emigrations from elsewhere. The need is obvious for genetic adaptation focused on the distinctive rapid rate of climate change. The selection and establishment of varieties and species in areas where they do not now grow must be undertaken. One means to these ‘artificial migration’ decisions involves use of future climate scenarios to determine the locations of current analogues for future conditions, followed by study and evaluation of the genetic resources at those current analogue locations. The number of assumptions needed to apply this approach is minimal, while the uncertainty in this approach involves the unique environmental conditions and ecosystem responses initially suggested above.

Keywords: rapid change, Holocene migrations, climate analogues, seed transport, regional extirpations

Plenary session – Day 1

GENOME-WIDE ASSOCIATION MAPPING: LESSONS FROM ARABIDOPSIS

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Although pioneered by human geneticists as a potential solution to the challenging problem of finding the genetic basis of common human diseases, genome-wide association studies have, owing to advances in genotyping and sequencing technology, become an obvious general approach for studying the genetics of natural variation and traits of agricultural importance. They are particularly powerful when inbred or clonal lines are available, because once these lines have been genotyped they can be phenotyped multiple times, making it possible (as well as extremely cost effective) to study many different traits in many different environments, while replicating the phenotypic measurements to reduce environmental noise. I will present results demonstrating the utility—as well as the limitations—of this approach in the model plant *Arabidopsis thaliana*.

Keywords: *Arabidopsis,* genome-wide association mapping, population genetics

ASPEN PHENOMICS: PHENOLOGY, HERBIVORY AND METABOLISM

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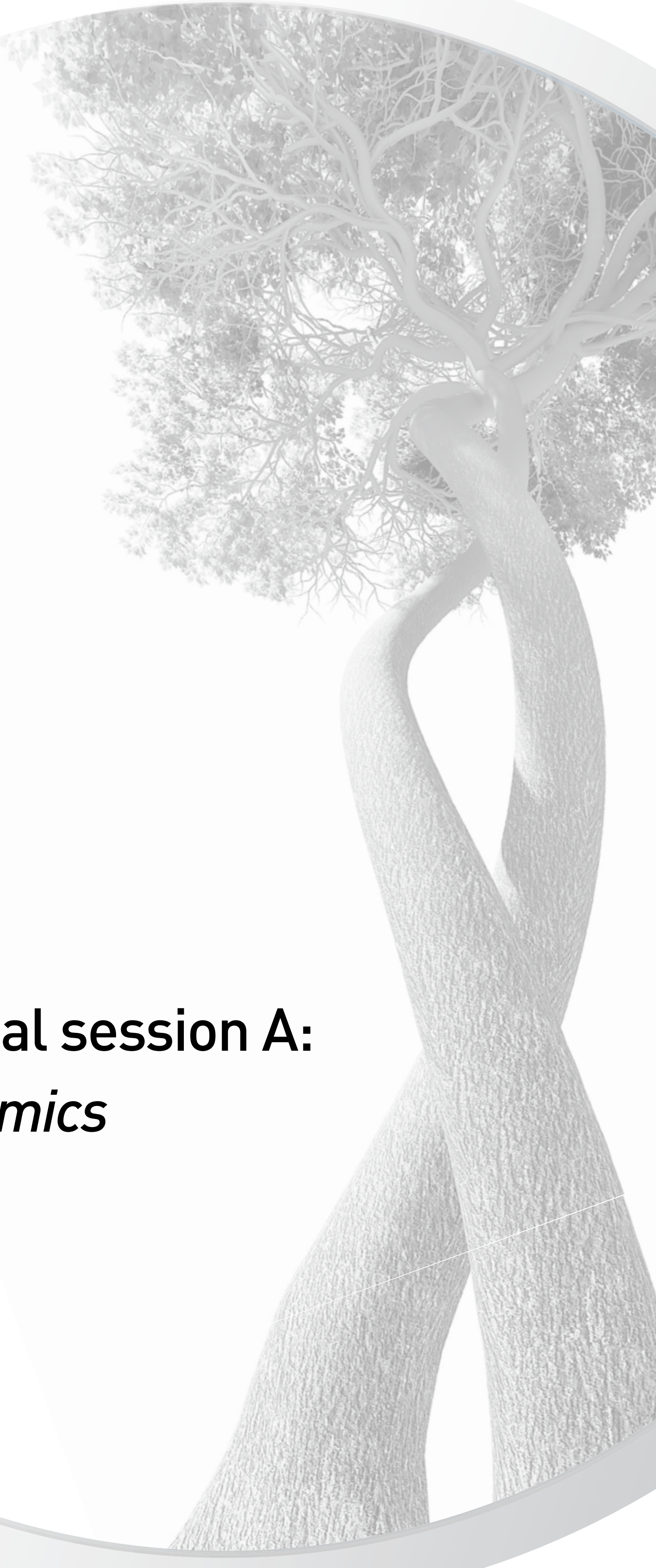
What sets the limit of tree distribution? In northern Europe, the ability to adapt to local seasonal changes is a key trait and correct autumn phenology is of crucial importance. We have studied how trees know it is autumn by investigating, among other things, gene expression, cell biology and natural variation, in particular in relation to autumnal senescence in European aspen *(Populus tremula* L.). We have shown that photoperiod is the key driver for autumn phenology traits, but have also studied the complex interaction between bud set and autumn senescence and demonstrated how temperature and the carbon and nitrogen balance of the tree influence the trait. In addition, association mapping using candidate genes (coding for photoreceptors and proteins involved in the photoperiodic pathway) and a collection of Swedish natural aspen accessions (the SwAsp collection) have identified a number of SNPs in several genes associating with variation in bud set or onset of in autumn senescence. We also study the relationship between the metabolic composition of aspen leaves and herbivore preferences. High-throughput analysis of leaf metabolites has been used to look at the dynamic nature of leaf metabolites, and also to classify aspen genotypes into distinct chemotypes in terms of phenolic glucosides, which are likely to explain variation in leaf herbivore preferences. The genes behind variation in these traits are not known. Finally, I describe how our resequencing of aspen genomes and transcriptomes and small RNAs may facilitate identification of the genes involved in regulating these traits.

Keywords: association mapping, genomics, metabolimics, phenology, *Populus tremula*, senescence



Parallel technical session A:

Population genomics

GENETIC SIGNATURES OF DEMOGRAPHICAL AND SELECTIVE EVENTS IN MEDITERRANEAN PINES

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Mediterranean forests are characterized by their spatial and temporal complexity and heterogeneity, and within this ecosystem pines exhibit keystone ecological and economic roles. Their presence in contrasting environments raises questions about their evolutionary history and the mechanisms involved in their adaptation. Recent advances in genomic tools have allowed the identification of a set of candidate genes that may be related to growth and fitness in these species. This may provide a new generation of 'adaptive' markers with application to conservation genetics and breeding. Here, we present two case studies based on candidate genes and wide sampling of natural populations of two Mediterranean pine species. In Aleppo pine (*Pinus halepensis* Miller), we first used coalescence simulations to fit demographical models and found an east-to-west colonization pattern, with lower nucleotide diversity in western populations (Spain and North Africa) than in eastern populations (Greece and Israel). We then conducted neutrality tests that suggest that the colonization of the western Mediterranean could have been accompanied by recurrent natural selection events. In maritime pine (*Pinus pinaster* Aiton), we used population genomic methods based on associations between environmental variables and genetic variation at candidate genes and performed neutrality tests within and among species. Different polymorphisms and haplotype variants of candidate genes were correlated with climate as well as differing from the null hypothesis for neutrality, suggesting the action of selection events. These two examples illustrate the power of different methods to detect the signature of selection in nature and the adaptive role of candidate genes, providing indispensable information for the application of markers in conservation and breeding programmes.

Keywords: candidate genes, demography, Mediterranean pines, selection

NATURALLY OCCURRING POLYMORPHISMS IN CANDIDATE GENES FOR PHENOLOGY IN BLACK POPLAR (*POPULUS NIGRA* L.)

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Natural populations of forest trees show adaptation to day length and seasons in their local environment. Timing of bud set is correlated with the latitudinal and elevational origin of the trees. Bud phenology traits are under strong genetic control in forest trees and show genetic differentiation along geographical clines. To investigate the molecular-genetic bases of such traits in black poplar (*Populus nigra* L.), we assessed the level and geographic distribution of genomic diversity in candidate genes coding for phenological characters. Based on the flowering time pathway in *Arabidopsis*, emphasis was given to 19 genes belonging to the phytochrome and cryptochrome families and the signal transduction pathway. Ninety-six alleles from 10 natural populations distributed across Europe were resequenced, and naturally occurring polymorphic nucleotide sites (SNPs) and short insertions/deletions (indels) were detected within the full-length sequence of those genes. A picture of the patterns of nucleotide variation was obtained, as well as the extent of the linkage disequilibrium. Based on these data, we discuss the evolutionary factors governing the variation detected and identify areas of the genome where natural selection has been especially important.

Keywords: genetic variation, linkage disequilibrium, nucleotide diversity, phenology, *Populus nigra*

MOLECULAR AND QUANTITATIVE GENETICS OF ADAPTIVE TRAITS IN TROPICAL FORESTS: ADAPTATION TO LOCAL ENVIRONMENTAL GRADIENTS IN THE GUIANA SHIELD

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Results on gene sequences and quantitative traits in tropical trees from several botanical families show large standing variation for adaptive traits and genes in these species. In particular, we have shown, by a common-garden approach, that in *Eperua* (Fabaceae) some quantitative traits related to plant physiology and growth are highly heritable and genetic values can change over very short distances, i.e. within few hundred metres, as a function of adaptive response to environmental gradients, in spite of gene flow. The same pattern is detected for gene sequences (for example for aquaporin genes), with differentiation (expressed by F_{ST}) in excess of 5% at the plot level when subpopulations are compared across the environmental gradient. Genes and quantitative traits vary also at the landscape and regional scale, but the correlation with large-scale environmental gradients is less clear, suggesting that for these species local adaptation to environmental gradients may be a major driver of genetic diversity. Reciprocal transplant experiments are helping elucidate the role of genotype × environment interaction in shaping genetic diversity patterns. The results are also discussed in the context of the interplay of interspecific gene flow and ecological divergence among closely related species with sympatric distribution, but having differential ecological preferences, as observed for quantitative trait and molecular divergence in *Symphonia* (Clusiaceae) and *Carapa* (Meliaceae).

Keywords: adaptive traits, environmental gradients, F_{ST}, tropical trees

GENOME SCANNING OF REGIONS SHOWING DIVERGENT EVOLUTION BETWEEN WHITE OAKS (*QUERCUS PETRAEA* (MATT.) LIEBL. AND *Q. ROBUR* L.): NEW INSIGHTS FROM THE OAK ALLELIC RESEQUENCING PROJECT

Pauline Garnier-Géré, Tiange Lang, Pierre Abadie, Thibaut Decourcelle, Valérie Léger, Jean-Marc Frigerio, Christian Burban, Catherine Bodénès, Erwan Guichoux, Cécile Robin, Grégoire Le Provost, Marie-Pierre Reviron, Marie-Laure Loustau, Rémy Petit, Christophe Plomion and Antoine Kremer

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The two white oaks, *Quercus robur* L. and *Q. petraea* (Matt.) Liebl., have a broad, largely sympatric distribution across Europe. Despite clear morphological and ecological differences, molecular analyses until recently showed very low differentiation between species at most nuclear markers. However, recent studies also indicate that several small genomic regions present much higher interspecific F_{ST} values. In order to identify and better characterize genomic regions potentially involved in divergent adaptation between these two species, a genome-scale project has been launched with the aim of resequencing 1000 DNA fragments from candidate genes potentially involved in adaptive differentiation (i.e. linked to drought-stress tolerance, hypoxia, reproduction, phenology, host–pathogen interactions) in a panel of 24 genotypes from both species sampled across their natural distribution. The strategy for producing the sequence data included the development of bioinformatics tools adapted to a non-model species, building on 100 000 assembled Sanger ESTs. Various approaches were used to test data quality and optimize the overall success rate. An automatic pipeline, SeqQual, for processing the data while integrating base quality was also developed with a focus towards population genetics analyses. The overall success rate was good (>80% of data recovered from the 1000 designed amplicons), and the number of SNPs ranged from 0 to more than 30 per amplicon. Genomic fragments of up to 2 kb were recovered and half the amplicons presented introns of variable lengths. A preliminary estimate of nucleotide differentiation (N_{ST}) between species on a set of ~100 reference amplicons (randomly chosen across the unigene) was higher (13%) than previous estimates based on SSRs and anonymous DNA markers, with values ranging from 0% to 60%. Distribution of nucleotide diversity in both species and differentiation between them are compared across all genes and between groups of genes linked to different biological functions. A search for genes showing higher differentiation than expected under neutral evolution will be performed by applying model-based genomic scans. Perspectives for using the genomic resources now available are discussed.

Keywords: adaptive differentiation, F_{ST}, N_{ST}, *Quercus robur, Quercus petraea*

INTERSPECIFIC CROSS-COMPATIBILITY BETWEEN TWO OAK SPECIES AND NUCLEOTIDE DIFFERENTIATION IN GENES POTENTIALLY INVOLVED IN REPRODUCTIVE BARRIERS

Pierre Abadie, Guy Roussel, Tiange Lang, Rémy Petit, Antoine Kremer and Pauline Garnier-Géré

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Despite large morphological and ecological divergence, pedunculate oak (*Quercus robur* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) can hybridize with each other and show very low molecular differentiation (nuclear and cpDNA markers, mean G_{ST} of around 3%). However, local 'hotspots' of genetic differentiation have been shown along the genome. These observations can be related to Wu's mosaic model of sympatric speciation at the genome level, and suggest the existence of 'speciation genes' that maintain species divergence despite gene flow. The aim of this study was to better understand the interaction between gene flow and divergent selection in these two oak species by focusing on interspecific reproductive barriers. To quantify hybridization levels, controlled crosses were performed between several pedunculate and sessile oak genotypes, and several traits relevant to crossing compatibility were measured (cytological observations of pollen tubes in styles, fructification, progeny viability). Comparison of inter- and intraspecific crosses revealed the existence of pre-zygotic barriers to hybridization. Significant variation was observed among oak genotypes across the interspecific crosses ($P<0.05$ on pre- and post-zygotic variables), suggesting a genetic influence on hybridizing compatibility. To study potential speciation genes, 27 homologues of candidate genes with functions linked to reproductive barriers (cross compatibility, flowering phenology) were chosen from model species databases. These genes were sequenced in both oak species, and their nucleotide diversity patterns were analysed. Mean nucleotide differentiation (N_{ST}) between species was higher (although not significantly) for the candidates (16.6%) than for 140 randomly chosen genomic fragments (13.2%), whereas nucleotide diversity (θ_π) was significantly higher ($P<0.001$) in the candidates genes (4.32e-03). Four genes potentially involved in reproductive isolation (cross-compatibility receptors or flowering transition) showed an interspecific differentiation greater than 50%, suggesting that they might have been subjected to divergent selection.

Keywords: controlled crosses, hybridization, nucleotide differentiation, *Quercus robur*, *Quercus petraea*

POPULATION GENOMICS OF PHENOLOGICAL CLINES IN SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LEIBL.)

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Clinal variation of phenological traits is frequently reported in provenance tests of forest trees. Congruent clines were identified across species, suggesting that they witnessed systematic directional selection in response to identical environmental gradients. We monitored nucleotide diversity in sessile oak (*Quercus petraea* (Matt.) Leibl.) along altitudinal and latitudinal gradients, along which clinal variation was observed for apical bud phenology. The study was based on 105 candidate genes that exhibited earlier differential expression during the transition from dormant to flushing buds. SNP discovery in the candidate genes was implemented through resequencing and data mining in EST sequence libraries. In total 384 SNPs were scored in 32 different sessile oak populations. Twenty-one populations were sampled along a latitudinal gradient in western Europe and 12 populations were sampled along an altitudinal gradient in the Pyrenees. We used various methods to detect SNPs deviating from neutral expectations and assessed their allelic frequencies along the altitudinal or latitudinal gradient. The overall differentiation assessed over all SNPs was of a similar magnitude to that usually reported for microsatellites. However, a few genes deviated from neutral expectations and showed congruent responses in the two clines, but not at the same SNPs. Clinal variation of allelic frequencies along latitudinal or altitudinal gradients was observed only rarely. Genetic association with phenotypic records of bud burst is currently under investigation and the results will be presented at the meeting. The results of the population surveys along environmental clines will also be compared with data coming from mapping pedigrees. Map position of SNPs will be compared to QTL of bud phenology. This comparative analysis will identify genes that can be used for comparative population genomics of clines in other Fagaceae species.

Keywords: clines, genetic differentiation, phenology, *Quercus*

IDENTIFICATION OF CANDIDATE GENES FOR BUD SET CONTROL IN NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.)

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Bud set and bud burst show strong latitudinal gradient over the natural distribution range of Norway spruce (*Picea abies* (L.) H. Karst.) and several other boreal tree species. There is now substantial evidence that differences in photoperiod response between populations can explain a large part of this observed gradient for bud set. Using both expression data and homology to model plants, we have identified a set of candidate genes for the photoperiodic control of bud set in Norway spruce. In order to elucidate the role of these genes in the observed difference in bud set, we sequenced these genes and a number of randomly chosen genes in individuals sampled from the natural distribution range of Norway spruce. Using approximate Bayesian computation we inferred and compared three demographic models and used simulations under the inferred models to evaluate the role of selection at candidate genes. One gene, which shows similarity to key circadian clock genes from model plants, showed a highly positive Tajima's *D,* deviating from all inferred demographic scenarios.

Keywords: demography, Norway spruce, nucleotide diversity, *Picea abies*, selection

ASSESSING PREVIOUS EVIDENCE OF LOCAL ADAPTATION IN A CRYOPHILIC PASTURE PERENNIAL USING TRANSFERABLE EST-SSR MARKERS

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Pastures above the timberline in Mediterranean high mountain ranges constitute one of the habitats most vulnerable to predicted climate change in southern Europe. *Silene ciliata* Pourret is a small, perennial plant that inhabits such pastures. A previous study using reciprocal sowing found evidence for local adaptation across an altitudinal gradient in the Sierra de Guadarrama (Madrid, Spain). The present study assessed the genetic structure of populations across altitudinal gradients on two adjacent mountains, and further assessed the strength of population structure. We tested 46 microsatellite markers located in transcribed genes (EST-SSRs) from floral tissues of *Silene latifolia* for use in *Silene ciliata*. Six had good signal quality and were polymorphic in *Silene ciliata*. These revealed high inbreeding coefficients (F_{is} values between 0.31 and 0.59), which could be related to limited pollination range and/or low seed dispersal, and the possible presence of null alleles, potentially associated with the process of marker transfer between species. Populations differed between mountains but not across altitudinal gradients. The low differentiation across the altitudinal gradient suggests the existence of significant gene flow among populations, which could be operating against local adaptation. The EST-SSR markers used may be unable to detect adaptive genetic differentiation among populations if they are not linked to genes underlying such variation. Furthermore, local adaptation may have arisen as a result of very recent selection forces and there may have not been enough time to produce changes in the genetic structure across the genome. Additional analyses with microsatellites isolated from *Silene ciliata* are now being implemented to complete the results obtained in this study.

Keywords: altitudinal gradient, EST-SSR, local adaptation, *Silene ciliata*

POPULATION GENOMIC STUDIES IN A WIDESPREAD CONIFER: NEUTRAL AND ADAPTIVE PROCESSES IN THE CONTEXT OF CLIMATE CHANGE

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Postglacial migration involves repeated colonization of novel environments, which leaves both neutral and adaptive signatures in the genome. We employed demographic modelling and association genetics to disentangle these effects in the widely distributed North American conifer, Sitka spruce (*Picea sitchensis* (Bong.) Carrière). Approximately 200 candidate genes were resequenced in a panel of 24 individuals sampled from across the ~3500 km latitudinal range of the species. The frequency distribution of mutations co-varied with latitude, as evidenced by clinal variation in Tajima's D ($R^2 = 0.84$, $P = 0.007$) and Fay and Wu's H ($R^2 = 0.65$, $P = 0.033$). We employed approximate Bayesian computation to evaluate a range of demographic models that might explain this result. When historical bottlenecks of varying age were simulated, distance of populations from the southern refugia explained most of the variation in bottleneck timing among populations ($R^2 = 0.89$, $P = 0.003$). These data suggest that sequential population bottlenecks during postglacial recolonization have resulted in diverse signatures among populations within the contemporary site frequency spectrum in Sitka spruce, with rare variants more common in the south, and medium-frequency variants more common in the north. To identify specific loci involved in adaptation to climate, we genotyped 768 SNPs in a large mapping population, for which two adaptive traits—autumn cold hardiness and budset timing—were also scored. After adjusting for the effects of neutral population structure using a cohort of 98 putatively neutral SNPs, we detected significant genotype–phenotype associations for 35 SNPs. Most notable among these were SNPs within five genes putatively involved in light signal transduction, the key pathway regulating autumn growth cessation in perennials. Finally, we employed the decision-tree algorithm 'Random Forest', which revealed an additional cohort of SNPs with predictive value that likely reflect the effects of epistasis. This study represents a significant step toward the goal of characterizing the genomic underpinnings of adaptation to local climate in conifers, and provides a substantial resource for breeding and conservation genetics in a changing climate.

Keywords: association mapping, approximate Bayesian computation, clinal variation, epistasis, *Picea sitchensis*

CANDIDATE GENE VARIATION IN COMMON BEECH (*FAGUS SYLVATICA* L.) ALONG AN ALTITUDINAL GRADIENT

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Beech (*Fagus sylvatica* L.) forests cover about 12 million hectares in Europe. Beech wood is much appreciated worldwide and many beech forests are regularly harvested for timber. Apart of its economic importance, European beech also has a major role in soil preservation and water cycles, which makes this species a target of ecologically oriented conservation. Climate change will force beech populations to adapt *in situ*, to migrate to higher latitudes or altitudes, or to rely on plasticity. This study is part of a project investigating variation in candidate genes related to cold and drought tolerance in *Fagus sylvatica* along an altitudinal gradient on Mont Ventoux, southern France. About 40 000 ESTs, sequenced within the European Network of Excellence EVOLTREE, have been processed to select candidate genes. About 200 primer pairs were designed and tested for amplification and polymorphism. Polymorphisms at the selected genes have been genotyped to estimate level and distribution of diversity within a population sampled along an altitudinal gradient and to dissect the role of selective pressures and demographic dynamics in European beech.

Keywords: beech, candidate genes, signature of selection, SNPs

Parallel technical session B: *Phenomics under climate change*

COMPARATIVE MAPPING IN SALICACEAE: A TOOL FOR IDENTIFYING IMPORTANT GENES CONTROLLING ADAPTIVE TRAITS

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Comparative mapping of QTLs for adaptive traits among populations and species can lead to the identification of genomic regions conserved over a long evolutionary period and therefore potentially harbouring genes of great importance for adaptive processes. At the same time, non-conserved QTLs could be responsible for local adaptation or interspecific differentiation. The availability of *Populus* as a model system for tree and woody perennial plant biology has largely been driven by the rapid development of genomic and molecular biology resources for this genus, culminating in the completion of a draft sequence of the genome of black cottonwood (*Populus trichocarpa* Torr.&A.Gray.). Numerous genetic maps are available in various *Populus* and *Salix* species and QTLs for adaptive traits (such as phenology and disease resistance) and sex have been mapped. All these maps showed a very good synteny with the *P. trichocarpa* genome. However, very few markers are currently shared between these maps. The objective of our study is to take advantage of the information on the *P. trichocarpa* genome sequence to perform QTL projection and identify candidate genes. Genetic maps from seven pedigrees belonging to four *Populus* spp. were aligned with the help of common simple sequence repeat and gene markers. Three additional published maps from *Salix* spp. were partially anchored to the *P. trichocarpa* genome sequence. QTL meta-analysis and projection of QTL intervals on the genome sequence were performed when sufficient genome-anchoring markers were available. These analyses allowed identification of large genome regions containing several hundred candidate genes. Using biological knowledge accumulated in public databases (e.g. Gene Ontology) and high-throughput enrichment tools, we have compared the list of genes contained in QTL regions with the entire genome gene content in order to identify enrichment in some functions. For some traits, we have identified significant gene ontology terms that are biologically significant.

Keywords: candidate genes, genetic mapping, *Populus* spp., QTL, *Salix* spp.

IS WATER USE EFFICIENCY A USEFUL AND RELEVANT TRAIT FOR ADAPTATION TO LOCAL CONDITIONS AND FOR BREEDING? PRESENT KNOWLEDGE AND RESEARCH NEEDS

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Differences in water use efficiency (WUE) have been extensively investigated in trees using carbon-13 in leaf biomass as a surrogate. We now have evidence of considerable diversity for this trait in several tree species, and valuable insight into the genetic control of this diversity is available. Nevertheless, two main questions remain open: 1) Is the observed diversity of WUE in natural populations neutral, or is WUE subject to selective pressure? 2) Is enhanced WUE a valuable target for breeding in plantation species such as poplar or pines? Based on recent evidence obtained in natural populations of oaks and beech, as well as in clonal plantations of poplar, we discuss these two questions and provide some answers, as well as outline further research needs. Briefly, there is currently little evidence from natural populations that the observed genetic diversity in ^{13}C discrimination and WUE is organized along clear patterns related for instance to soil water availability. We therefore urgently need more data on the genetic variability in ^{13}C discrimination at local scales, i.e., common garden experiments. Breeding for enhanced WUE will not produce genotypes with enhanced drought resistance, but could possibly increase the sustainability of plantation forestry in regions where water availability is a major issue.

Keywords: ^{13}C, functional trait, isotopic discrimination, transpiration efficiency, water availability

A HOLISTIC VIEW ON THE EFFECT OF LONG-TERM DROUGHT STRESS AND REWATERING IN OAK

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Over the coming decades Europe's climate is expected to change, with longer drought periods during the summer months. Trees able to adapt easily to longer drought periods will be needed for sustainable forest management and afforestation. This study investigated the effect of long-term drought stress and rewatering on young pedunculate oak trees (*Quercus robur* L.), the goal being the development of functional markers for the selection of trees with increased drought resistance from natural populations. Four approaches were combined to identify informative markers. In a 2-year glasshouse experiment, 5-year-old oak clones originating from tissue culture were subjected to controlled drought stress conditions in a closed environment. Three treatments were applied: 1) control, 2) one year drought followed by one year rewatering and 3) two years of drought. During the course of the experiment phenotypic parameters were monitored and plant material was sampled at regular intervals for analyses on the physiological, transcriptome (cDNA microarray with 18 545 unique genes) and proteome level. Oaks exposed to drought stress displayed reduced growth and change in flushing time, osmotic adjustment and starch degradation, reinforcement of the cuticula, repression of photosynthesis, and induction of genes with protective function as well as genes associated with senescence. Rewatering resulted in a recovery of photosynthesis, repression of genes with protective function and senescence-associated genes, induction of growth and cell-wall-related processes, and the induction of rehydration-specific genes. On the basis of these results, suitable functional markers will be developed for the selection of oaks with increased potential to withstand drought.

Keywords: cDNA microarray, drought, *Quercus robur*, rewatering

HOW WILL NATIVE BLACK POPLAR (*POPULUS NIGRA* L.) BE INFLUENCED BY DROUGHT IN A FUTURE EUROPE? EVIDENCE FROM GENETIC, GENOMIC AND PHENOTYPIC STUDIES

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Predictions indicate that summer droughts will increase in frequency and intensity across Europe as a consequence of global climate change. Therefore it is important to study the adaptation to drought of black poplar (*Populus nigra* L.), which is both ecologically and economically important in Europe. Wild black poplar trees were collected for an association genetics study in five European countries, from Spain to The Netherlands, reflecting a wide range of rainfall and temperatures. These genotypes were grown in a common garden in Belgium under well-watered conditions. Carbon isotope discrimination, biomass and leaf size varied with their population of origin. Small leaf size was observed in genotypes from Spain and southern France, and is considered a possible adaptive trait for drought tolerance, as it reduces water loss. Six genotypes from a range of latitudes of origin and displaying contrasting leaf phenologies and carbon isotope discriminations were then selected for a drought experiment under controlled conditions. Carbon and oxygen isotope discrimination, stomatal conductance, biomass and leaf traits were measured to identify physiological differences in large- and small-leaf genotypes in response to drought. Leaf samples were also collected 20 days after drought and RNA was extracted for microarray analysis and transcriptomic studies. Direct comparisons between the transcriptome of extreme genotypes in well-watered and drought conditions provide insight into the genomic pathways induced during water deficit. These results provide important insights in genetic variation underpinning adaptation to drought across Europe. Such variation may be valuable in future conservation and management of trees in the face of climate change.

Keywords: carbon isotope discrimination, drought, *Populus nigra*

PHENOTYPIC AND MOLECULAR PLASTICITY OF TWO EUCALYPTUS CLONES IN RESPONSE TO WATER DEFICIT

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In the context of climate change, water availability will be the main limiting factor affecting biomass production, in particular in intensively managed forest tree plantations. The complexity of drought stress response calls for studies that combine physiological, anatomical and molecular investigations at different scales ranging from particular cells to the whole plant. Such a strategy was developed for two eucalyptus clones used in industrial plantations in the Republic of the Congo: one hybrid of *E. urophylla* × *E. grandis*, and one hybrid obtained from open-pollination of *E. platyphylla*. These two genetic units, known to differ in their water use efficiencies, were submitted to two watering regimes (irrigated vs non-irrigated during the dry season) in open field conditions and monitored over 18 months. First, molecular plasticity of the two genotypes was investigated at the transcriptome level, following two strategies. Using high-throughput sequencing (454-Roche) we found 2720 contigs showing genotype, treatment or genotype by treatment interaction effects. The expression of 50 candidate genes (selected according to their expression pattern and their ontology) was characterized from several tissues and for different development stages by qRT-PCR. Second, phenotypic plasticity was assessed for various ecophysiological (biomass, delta^{13}C and architectural development), anatomical (leaves and stem structure) and chemical (component characterization of leaves by HPLC) traits. The combined analysis of reaction norms of candidate genes and phenotypic traits is being investigated and should reveal co-variations between traits and expressed genes. This study should eventually improve our understanding of the molecular mechanisms involved in the genetic control of water deficit resistance in *Eucalyptus* spp. Finally, this study will supply relevant candidate genes and/or traits to screen the genetic diversity of natural and breeding populations to estimate their adaptive potential.

Keywords: eucalyptus, phenotypic plasticity, water deficit, 454 sequencing

OZONE POLLUTION COMPROMISES *POPULUS* TRANSCRIPTOME RESPONSES TO INSECT HERBIVORE ATTACK

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Ozone is considered one of the most severe air pollutants in forest ecosystems around the world, particularly downwind of industrialized areas. In *Populus* species and hybrids, ozone causes various symptoms of oxidative stress. Similar signalling pathways may be activated in response to both biotic and abiotic stresses; therefore, we used genomic resources of *Populus* to investigate the influence of ozone pollution on plant responses to herbivore attack. In separate experiments, we used three genotypes of hybrid *Populus* that varied in their susceptibility to ozone pollution. Experiments were conducted in Continuously Stirred Tank Reactor fumigation chambers. We used ozone concentrations of 80 ppb to reflect spring and summer conditions in central Pennsylvania. Half of the plants were in control chambers where no ozone was added. After one week, all plants had portions of their stems enclosed in soft mesh bags and gypsy moth (*Lymantria dispar* L.) larvae were added to half of the bags. After 24 hours, leaves with similar amounts of damage and undamaged controls were collected and subjected to microarray hybridizations using either a custom array containing approximately 6500 unigenes or a commercially available full genome array. Ozone exposure severely compromised the dynamic responses to insect herbivore feeding. While approximately 20% of the *Populus* transcriptome was responsive to herbivory under control conditions, only approximately 4% of the transcriptome responded to herbivory after 80 ppb ozone. Moreover, the magnitude of expression of the genes under ozone exposure was mostly lower than under control conditions. Of particular interest, expression of genes associated with defence signalling and induced resistance was compromised or strongly reduced under ozone exposure. Consistently, concentrations of signalling compounds and defence metabolites were significantly reduced despite extraordinary accumulation of fatty acid precursors resulting from ozone-mediated destruction of cell membranes. We will discuss our results in the context of this study and other related work that shows effects of ozone pollution.

Keywords: herbivory, hybrid poplar, microarray, ozone, *Populus*

ADAPTATION OF FOREST TREES TO A HIGH CO_2 WORLD: THE TRANSCIPTOME FOR DELAYED AUTUMNAL SENESCENCE

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Over recent decades extensions to the growing season have been measured at the continental scale using remote sensing and at the plant scale using observations such as bud-burst, bud-set, leaf colour change and leaf drop. While a strong association exists between rising global temperatures and an earlier spring, the association between temperature and the later on-set of autumnal senescence is weak. We hypothesized that an increasing atmospheric carbon dioxide (CO_2) concentration may partly account for delayed autumnal senescence and tested this using the EUROFACE (www.unitus.it/euroface) facility, a 9-hectare experimental plantation of poplar trees growing in central Italy. We investigated the phenomenon using two large free air CO_2 enrichment (FACE) experiments and three species of poplar (*Populus nigra* L., *P. ×euramericana* Guinier and *P. tremuloides* Michx.). Experimental plots within the EUROFACE plantation expose trees to a CO_2 concentration of 550 μmol mol^{-1}, the level predicted in 2050 and 42% above current levels. Whole canopy remote sensing of normalized difference vegetation index (NDVI), changes in leaf area index (LAI) and leaf chlorophyll content were all measured to quantify the effect CO_2 has on the rate of autumnal senescence. It was estimated that elevated CO_2 concentration delayed 50% canopy leaf loss by ca. 10 days. To gain mechanistic insight, the PICME microarray was used to determine differential gene expression between leaves senescing in either ambient (a[CO_2]) or elevated CO_2 concentration (e[CO_2]) during this FACE experiment. Leaf anthocyanin and carbohydrate contents were also measured. Leaf biochemical pathways for secondary metabolism and glycolysis were significantly up-regulated by elevated CO_2 levels during senescence, particularly those related to anthocyanin biosynthesis. Expressed sequence tags (ESTs) representing the two most significantly up-regulated transcripts under elevated CO_2 levels, *LDOX* (leucoanthocyanidin dioxygenase) and *DFR* (dihydroflavonol reductase), both down stream in anthocyanin biosynthesis, gave (e[CO_2] / a[CO_2]) expression ratios of 39.6 and 19.3, respectively. In line with the growth difference balance hypothesis we propose that elevated CO_2 stimulates secondary metabolism, namely anthocyanin biosynthesis, during senescence, which prolongs leaf longevity.

Keywords: autumnal senescence, anthocyanin biosynthesis, cDNA microarray, elevated CO_2, *Populus*

DIFFERENTIAL GENE AND MICRORNA EXPRESSION RELATED TO AN EPIGENETIC MEMORY AFFECTING CLIMATIC ADAPTATION IN NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.)

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Norway spruce *(Picea abies* (L.) H. Karst.) displays a temperature-dependent epigenetic memory from the time of embryo development, which thereafter influences the timing of bud phenology. As a first step toward unravelling the molecular mechanism behind an epigenetic memory, transcriptional analysis was performed on seedlings from seeds of six full-sib families produced under cold (CE) and warm (WE) embryogenesis temperature regimes. We prepared two suppressive subtracted cDNA libraries, representing genes predominantly expressed after bud set induction in plants from seeds obtained after CE and WE embryogenesis. Sequencing and annotation revealed considerable differences in the transcriptome of WE and CE seedlings. We studied the expression patterns of 32 selected candidate genes using qRT-PCR. Five genes, two transposon-related genes and three with no matching sequence in databases showed differential expression in progeny from CE and WE correlated with family differences. Another step was to study microRNAs (miRNAs), which are endogenous small RNAs exerting epigenetic gene regulatory effects. We tested for their presence and differential expression. We then prepared concatemerized small RNA libraries from seedlings of two full-sib families, originated from seeds developed in a cold or a warm environment. One family showed distinct epigenetic effects whilst the other did not. Sequencing identified 24 novel and 4 conserved miRNAs. Further search and screening of the conserved miRNAs confirmed the presence of 17 additional miRNAs. Most of the miRNAs were targeted to unknown genes. The expression of seven conserved and nine novel miRNAs showed significant differences in transcript levels in the full-sib family showing distinct epigenetic difference in bud set, but not in the non-responding full-sib family. The differential expression of specific miRNAs indicates their putative participation in epigenetic regulation. Putative miRNA targets were studied. These findings may contribute to our understanding of the epigenetic mechanisms underlying adaptive changes acquired during embryogenesis in Norway spruce.

Keywords: bud set, epigenetic, gene expression, microRNA, *Picea abies*, RT-PCR

PLANT REMODELLING BY MANIPULATION OF FLOWERING TIME IN TREES

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Coordinated flowering is very important for successful reproduction of forest tree species adapted to local environments. However, changing climatic conditions can lead to a shift of flowering time, endangering the long-term survival of tree populations. Research into flowering time requires natural mutants and transgenic lines with differing flowering phenotype. Unfortunately, very few early-flowering mutants are available for forest tree species. One known natural early-flowering mutant is the Acrocona Norway spruce, *Picea abies* (L.) H. Karst. cv 'Acrocona'. This mutant is characterized by precocious formation of terminal cones. However, the nature of the mutation still remains unknown. Key regulators of floral induction have been studied extensively in the plant model species *Arabidopsis thaliana* (L.) Heynh. and, to some extent, in poplar (*Populus* spp.). In *Arabidopsis*, *SOC/FUL* (flowering time MADS-box genes) double mutants are, surprisingly, characterized by wood formation and perennial growth, indicating involvement of these two genes in other developmental processes. Heterologous over-expression or RNAi inhibition of *Arabidopsis* flowering-time control genes in poplar can represent an alternative for studying tree growth and tree development. The meristem identity gene *LEAFY (LFY)*, for instance, induces precocious flowering in transgenic poplar. Side effects of this gene are dwarfism, single flowers (instead of catkins) and occasionally gender change in male clones. The *FLOWERING-LOCUS-T (FT)* gene also induces early flowering in poplar. Poplar plants transformed with *FT* show a more normal phenotype than those transformed with *LFY*. The homeobox gene *BpMADS4 (FUL* homologue) induces early flowering in birch and apple but not in poplar. However, this gene induces an extremely delayed dormancy in poplar. Over-expression and RNAi-inhibition experiments using *Arabidopsis* flowering-time genes *SOC, FUL* and *FPF* are ongoing in poplar. Their role in the induction of flowering and wood formation properties in poplar will be assessed.

Keywords: climate change, flowering-time control gene, poplar, tree development

TROPHINOAK: A CONTROLLED SYSTEM WITH OAK MICROCUTTINGS TO STUDY GENE EXPRESSION AND RESOURCE ALLOCATION IN MULTITROPHIC INTERACTIONS

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Plant functioning depends on biotrophic interactions affecting internal processes, such as endogenous rhythmic development with alternating shoot and root growth flushes in oak trees (*Quercus robur* L.). In an interdisciplinary project (www.TrophinOak.de), six groups will compare gene regulation and resource allocation in oaks interacting with six different biotrophic partners: an ectomycorrhizal fungus (*Piloderma croceum* J. Erikss. & Hjortstam), a root pathogen (*Phytophthora quercina* T. Jung), a root feeding nematode (*Pratylenchus penetrans* (Cobb) Chitwood & Oteifa), a rhizosphere consumer (Collembola: *Protaphorura fimata*), a mycorrhization helper bacterium (Streptomyces AcH505) and a leaf pathogenic fungus (*Microsphaera alphitoides* Griffon & Maubl.). A common 'joint experimental platform' consisting of microcuttings of one defined oak clone with and without mycorrhizae will be used to investigate the influence of the biotrophic partners under standardized conditions. Tracer experiments will be conducted with ^{13}C and ^{15}N and transcriptomics at two plant developmental stages using a 30 000 EST/oligo microarray (PICME Oak) optimized in the EVOLTREE project in collaboration with the Austrian Institute of Technology in Seibersdorf and INRA at Bordeaux, France. Additional experiments investigate the combined effects of individual biotrophic partners on the dynamics of the associations, above-ground–below-ground interactions and modifications by changing climatic conditions. The project aims at disentangling regulation mechanisms in interactions between plants and biotrophic partners, initiating controlled multitrophic experiments and constructing a thematic microarray. Follow-up research will include studies of gene regulation and field analyses on the impact of multiple biotrophic partners on performance of clonal oak trees released in the field and used as 'phytometers' along climatic gradients in the four Helmholtz observatory sites of TERENO (www.tereno.net). Expression regulation of specific genes involved in the biotrophic associations will be examined under a range of environmental conditions.

Keywords: C allocation, gene expression, multitrophic interactions, N allocation, *Quercus robur*, root–shoot flushing

Plenary session – Day 2

ECOLOGICAL AND PHYLOGENETIC LIMITS OF ADAPTATION IN FOREST SEDGES

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The bulk properties of ecological communities, such as species abundance and diversity, can be predicted by neutral theory. At the same time, it is obvious that different habitats often support characteristically different suites of species and that distantly related species often occupy different habitats. Hence, neutral theory, if it applies at all, must break down at some level of ecological difference and phylogenetic distance, beyond which community composition is determined by functional divergence. We have mapped the limits of neutrality for a rare example of tropical diversity in a temperate setting, the genus *Carex* (sedges), using a reliable and detailed phylogeny and fine-scale biological surveys of forest and fen sites in the temperate and boreal zones of Canada. The sedge communities of forest and fen scarcely overlap in species composition and are clearly phylogenetically aggregated. Within either habitat, sister species are not consistently similar and ecological similarity estimated from co-occurrence is unrelated to phylogenetic distance. Ecological similarity is, however, detected among clades of several or many species, and clades or sets of clades separated by an average of more than about 2% sequence divergence are often ecologically segregated, primarily in relation to drainage.

Keywords: *Carex*, communities, ecological segregation, fen, forest, neutral theory

ANALYSING GENETIC DIVERSITY WITH DEEP SEQUENCING: FROM SINGLE GENOME TO ENVIRONMENTAL GENOMICS

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Investigating biodiversity traditionally implies tools for collecting samples and rules for examining and for comparing their biological traits. In genetics, for decades the high cost of DNA sequencing led to consideration of only single individuals from a few species as genomic models. With recent progress in sequencing technologies, it is now possible to sequence many more individuals and populations at the genomic and even transcriptional level, revolutionizing genomic sciences. Work is being conducted on groups of species that remain poorly studied despite their ecologically crucial role, such as plankton. In this context, we are participating in Tara-Ocean, a multi disciplinary scientific project based on a circum-global expedition over all oceans over three years. We are assessing the impact of new generation sequencing on the feasibility of analysing the diversity of marine protists at the genomic and transcriptomic levels. The first phase of the project is to establish a set of methodologies enabling the assessment of the complexity of these populations, and to constitute a large collection of individual genome sequences from diverse phyla. We present the strategy and preliminary results indicating the relative impact of the different sequencing technologies on a survey of different cell sizes of Mediterranean plankton communities at different depths.

Keywords: bioinformatics, genomics, plankton, sequencing, Tara-Ocean

CONSTRAINTS ON GEOGRAPHIC RANGE CHANGE UNDER CLIMATE CHANGE AND POSSIBLE METHODS TO OVERCOME THEM

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There are several reasons why species may not shift their geographic distributions under modern climate change, including natural and anthropogenic dispersal constraints and local adaptation among populations within a species' range. Given the rapid rate of projected climate change within the next ~100 years, constraints on natural range change may require human intervention for species conservation and the maximization of ecosystem goods and services. I will discuss emerging evidence for the role of climate versus other factors in setting the range boundary of species and thus their sensitivities to climatic change. This discussion will focus on dispersal limitation and the likelihood that populations near the poleward range boundary will increase under climate warming. I also will discuss pros and cons of various possible intervention strategies that address these issues. No strategy is without risk and none will capture a large number of species or ecosystems at a single time. This implies considerable cost to biological adaptation to climate change (i.e., human intervention) and reiterates the importance of greenhouse gas emission reduction.

Keywords: climate change, dispersal, geographic range, greenhouse gas emission reduction

Parallel technical session C: *New technologies in ecosystem genomics*

ANALYSIS OF NATURALLY OCCURRING GENETIC VARIATION IN TREES USING NEXT-GENERATION SEQUENCING: BUILDING COMPREHENSIVE CATALOGUES OF VARIATION

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The genomics revolution of the last 15 years has improved our understanding of the genetic make-up of living organisms. Together with complete genomic sequences for an increasing number of species, high-throughput and parallel approaches are available for the analysis of DNA sequence variation, transcripts and proteins. The use of genomic tools has allowed us to start to unravel the genetic make-up of traits that are relevant to adaptation. At the same time, a deeper understanding of what natural variation is at the sequence level has also been achieved. The pace at which we can analyse natural sequence variation has recently been greatly accelerated thanks to the advent of new DNA sequencing technologies. The comparative sequencing of several plant genomes revealed that, in addition to single nucleotide polymorphisms (SNPs), transposable elements are largely responsible for extensive variation in both intergenic and local genic content not only between closely related species but also among individuals within a species. In addition, larger structural variants can be detected, similar to the copy number variants identified in the human genome and involving hundreds of thousands of base pairs of DNA and tens of genes. A single genome sequence may therefore not reflect the entire genomic complement of a species. This realization prompted us to introduce in plants the concept of the pan-genome, which includes core genomic features common to all individuals and a dispensable genome composed of non-shared DNA elements that can be individual- or population-specific. Here, we describe the variation that can be detected among tree genotypes using next-generation sequencing methodologies, not only as SNPs but also and especially as structural variants due either to simple transposable element insertions or to insertions or deletions of large genomic regions. We focus on how to create either horizontal catalogues of genetic variation, i.e. looking at the variation in a few very interesting genes in a very large number of individual trees, or vertical catalogues, i.e. looking at the variation in the entire genome of a few very interesting individuals, and provide examples of how to use such catalogues to address specific biological questions.

Keywords: next-generation sequencing technologies, structural variation, transposable elements

COMPARATIVE GENOMICS OF TROPICAL TREE SPECIES USING ASSEMBLY-FREE TECHNIQUES

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The genome has a phenotype. One phenotype is the 'finished' reference genome sequence determined by the degree of linkage among the sequenced elements. Short-read sequencing (SRS) breaks down virtually all of this linkage. While algorithms for *de novo* assembly of SRS are improving, much of the data remains unanalysed when no reference genome is available. Completing reference genomes for every species in diverse groups such as tropical trees would be prohibitively expensive. All attempts at assembly or alignment of these data rely upon a hash table or graph of the DNA sequence diversity and abundance. Here, we explore the direct analysis of the frequency table of k-mer fragments of SRS data, where k is much shorter than the read length. We use whole genome shotgun sequence from the Illumina platform for 17 tree species and examine three different macroevolutionary perspectives: 1) population genetics of an endangered tropical timber species; 2) generic level variation in tropical figs; and 3) family level variation in the Fagaceae. We demonstrate that phylogenies can be directly reconstructed and that the greater the genomic coverage the more accurate the tree. We also illustrate how historical incongruence can be dissected through the fractionation of genomic variation into the different possible groupings of study taxa. The genomic fractions that conflict with history but correlate instead with geographic or phenotypic variation can then be used for more intensive study and finishing. This approach opens avenues for the effective discovery of genetic elements associated with different ecologies and life histories and thus patterns of ecological adaptation and genomic diversification.

Keywords: alignment-free, *Ficus, Gonystylus, Lithocarpus*, phylogenomics

ULTRA-DEEP SEQUENCING OF THE NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.) NEEDLE TRANSCRIPTOME

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Due to both the economic and ecological importance of spruce species there have been several large projects devoted to characterizing their transcriptomes. The majority of efforts have, however, been undertaken in North American spruce species (*Picea sitchensis* (Bong.) Carrière and *Picea glauca* (Moench) Voss). There are still less than 15 000 expressed sequence tags for the European Norway spruce (*Picea abies* (L.) H. Karst.) deposited at Genbank. In order both to facilitate comparative studies between European and North American spruce species and to gain a better knowledge about the Norway spruce transcriptome we have sequenced more than 100 million short reads from a Norway spruce needle RNA library. Close to 50% of all obtained reads mapped against putative unique transcripts obtained from North American spruce species, highlighting the short evolutionary distance between spruce species. Of the almost 30 000 putative full length genes from *Picea glauca*, more than 10 000 were sequenced at 90% or more coverage and only around 5000 were not sequenced by even a single read. This is a first step in characterizing tissue specific transcriptomes in Norway spruce and will hopefully be useful in the upcoming annotation of the Norway spruce genome. It will, however, on a shorter time-scale be used to identify SNPs in expressed genes and in gene expression studies.

Keywords: conifer, next generation sequence data, transcriptome

A PIPELINE FOR MOLECULAR TAXONOMY

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Identifying the species of an organism from markers is classically implemented using BLAST (Basic Local Alignment Search Tool) libraries on huge external databases, such as GenBank. The process involves: 1) extracting all items in the database similar to marker sequences of the unknown species; 2) comparing the extracted sequences with the markers; and 3) building a possible phylogeny to identify the species. This process is convenient for a few queries at a time. However, studies are increasingly focusing on large collections of diverse individuals sampled at the community level, using high-throughput sequencing technologies and metagenomics. Some markers, especially those that are variable between species, do not permit alignment of large and diversified data sets, mainly because of homoplasy. To overcome this problem, we investigated a new procedure. We show how a better use of the algorithm underlying BLAST can be used directly, without multi-alignment, giving a sort of 'inner BLAST' of large data sets. A link with phylogenies will be presented. Some examples will be provided on trees and some other taxa (insects, fungi, algae). Some difficulties that require further research will be highlighted.

Keywords: bioinformatics, BLAST, molecular taxonomy, pipeline

LARGE-SCALE INTEGRATION OF DISTRIBUTED HETEROGENEOUS DATA SOURCES WITHIN EVOLTREE

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Very large amounts of heterogeneous information, data and knowledge are continuously being generated by EVOLTREE research activities. Additionally, similar existing datasets have been shared by partners or external collaborators. One major goal of EVOLTREE is to integrate all these distributed electronic resources into a single information system that is available to the project and the whole scientific community by: 1) developing thematic databases; 2) populating these databases with research results from all partners; and 3) setting up an Internet search portal over all thematic databases (vertical integration). This so-called eLab provides central access to various distributed and heterogeneous database systems all over Europe. It allows queries to be submitted on line to 12 databases currently linked to the eLab. The results are presented as a single result set, resolving overlaps and links in the underlying data automatically. Currently 33 mapping pedigrees, 8 association populations, georeferenced information about 3767 natural populations of oak, 22 microarray experiments, 1024 physical and QTL maps and 87 investigated phenotypic traits are part of the eLab information system. A BLAST service provides access to 1 883 118 EST, 305 815 nucleotide and 256 683 protein sequences from beech (*Fagus*), oak (*Quercus*), poplar (*Populus*), ash (*Fraxinus*), pine (*Pinus*), spruce (*Picea*), fir (*Abies*), fungi of the *Glomus* and *Laccaria* genera and *Lymantria* moths coming from the project and the NCBI database. Additional information about pedigrees, association populations and microarray experiments, as well as further EST sequences will be included in the near future. SNP and SSR data will be introduced into the system soon. Furthermore, additional databases will be linked to the eLab by mid 2010. The EVOLTREE web portal and the eLab are available at http://www.evoltree.eu.

Keywords: data integration, EVOLTREE

SEQUENCING THE GENOME OF CHINESE CHESTNUT (*CASTANEA MOLLISSIMA* BLUME) FOR ECOSYSTEM RESTORATION

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America's forests are facing severe and increasing threats from diseases, pests, invasive plants, pollution and climate change. These destructive forces lead to trees dying across millions of hectares every year, with mortality predicted to be as high as 25% within the next 15 years. The Forest Health Initiative (FHI) was created as a collaborative effort to advance the application of genomics, biotechnology and molecular genetics to rapidly address forest health problems. The loss of American chestnut (*Castanea dentata* (Marshall) Borkh.) due to the introduction of the exotic fungal pathogen *Cryphonectria parasitica* was one of the worst ecological disasters of the 20th century. The first target of the FHI is to restore native forest ecosystems by reintroducing American chestnut. Our goal within the FHI is to use genomics to identify pathogen-resistance genes for use in chestnut breeding and genetic transformation. We are developing a high-quality reference genome sequence for blight resistant Chinese chestnut (*Castanea mollissima* cv 'Vanuxem') using next-generation DNA sequencing technologies. We have obtained over 32 000 transcript contigs, including 874 full length coding sequences, in the NSF-sponsored project on Genomic Tools for the Fagaceae. These will facilitate identification of expressed genes in the assembled genome of Chinese chestnut. The highly dense genetic linkage map and physical map created by colleagues in the Genomic Tools for the Fagaceae and FHI projects will guide assembly of the genome sequence. To identify candidate genes for blight resistance, the reference genome sequence will be integrated with the physical, cytomolecular and genetic maps and compared with genomes of American chestnut and a resistant chestnut hybrid that we will produce by resequencing. We hope that our work will encourage the inclusion of genome sequencing projects in other forest ecosystem programmes.

Keywords: blight resistance, *Castanea*, chestnut, genome, sequence

BIOINFORMATIC ANALYSIS OF SANGER AND 454 ESTS IN OAK

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The Fagaceae family comprises about 1000 woody species, spread throughout the northern hemisphere. About half belong to the *Quercus* genus. These oaks are often a source of raw material for carpentry, furniture and cabinet-making, veneer, cask-making and fuel wood. Pedunculate (*Quercus robur* L.) and sessile (*Quercus petraea* (Matt.) Liebl.) oaks are among the most important deciduous forest tree species in Europe. Despite their ecological and economic importance, very few genomic resources have yet been generated for these species. Here, we describe the development of an EST catalogue that will support ecosystem genomics studies, where geneticists, ecophysiologists, molecular biologists and ecologists are brought together to understand, monitor and predict functional genetic diversity. We generated 145 827 read sequences from 11 un-normalized and nine subtractive cDNA libraries by the Sanger method. Unexploitable chromatograms and quality checking led us to eliminate 19 902 sequences. Pyrosequencing was also conducted for 14 un-normalized libraries, generating 1 948 579 read sequences, from which 370 566 sequences (19.0%) were eliminated, resulting in 1 578 013 reliable sequences. Following clustering and assembly using the TGICL software, 1 704 117 EST sequences collapsed into 69 154 tentative contigs and 153 517 singletons, providing 222 671 non-redundant sequences (including alternative transcripts). Gene ontology annotation was then assigned to 29 303 unigene elements. Blast search against the SwissProt database revealed putative homologues for 32 810 (14.7%) unigene elements, but more extensive search with Pfam, Refseq_protein, Refseq_RNA and eight gene indices revealed homology for 67.4% of them. The EST catalogue was examined for putative homologues of candidate genes involved in bud phenology, cuticle formation and phenylpropanoid biosynthesis. Our results suggest a good coverage of genes involved in these traits. Simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) were also searched, resulting in 16 358 SSRs and 36 411 putative SNPs. All of the results are available at the Oak Contig Browser (http://genotoul-contigbrowser.toulouse.inra.fr:9092/*Quercus*_robur/index.html) with user name <oak> and password <*Quercus*33>. This genomic resource provides a unique tool for discovering genes of interest, studying the oak transcriptome and developing new markers to investigate functional diversity in natural populations.

Keywords: bioinformatics, oak, Sanger, unigene, 454

IN SILICO SCREENING OF CONSERVED ORTHOLOGOUS SEQUENCES (COS) FROM FAGACEAE ESTs RESOURCES

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Conserved orthologous set (COS) markers are single or low-copy-number genes in a set of plant genomes and have been developed for many plant species as the anchor markers for evolutionary genomics studies and comparative mapping. The lack of comparative whole genome analysis and molecular markers in the Fagaceae family (e.g. *Quercus* [oak], *Castanea* [chestnut] and *Fagus* [beech]) hamper tree improvement as well as the link between the physical map and genetic maps. Traditional COS marker development was focused on the one-to-one relationship between *Arabidopsis thaliana* and the target species. Advances in plant genome sequencing projects provide the opportunity to build COS markers based a broader phylogenic coverage. Here we present the development of markers derived from various Fagaceae EST sequencing projects. The oak, chestnut and beech EST data were generated from various tissues and projects/funding sources (EU-EVOLTREE and NSF-Fagaceae project) and clustered into 36 883, 37 531 and 15 754 unigenes, respectively. Six sequenced dicot (*Arabidopsis thaliana, Arabidopsis lyrata*, black cottonwood [*Populus trichocarpa* (Torr. & Gray)], barrel medic [*Medicago truncatula*], soybean [*Glycine max*] and grapevine [*Vitis vinifera*]) genomes also were included in the whole genome comparison. We used the TribeMCL clustering algorithm to identify the homologous sequences and created 18 750 multigene families. We gradually broke down the COS markers based on the evolutionary constraint, with the most stringent COSs having exactly one copy of orthologous sequence in *A. thaliana, A. lyrata, P. trichocarpa, M. truncatula*, *G. max* and *V. vinifera* and less stringent COSs which considered only low-copy-number unigenes in oak, chestnut and beech. In total, we identified 15 categories and 6108 COS markers. These COS markers provide the framework for understanding the synteny within the Fagaceae family. The Illumina SNP array is under construction and will be verified on the mapping population.

Keywords: conserved orthologous set markers, unigene

Parallel technical session D:
Eco-regional trends in adaptation

HISTORY AND EVOLUTION IN THE SCOTTISH SCOTS PINE (*PINUS SYLVESTRIS* L.) POPULATION

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From a postglacial high of around 15000 km^2, the Scottish population of Scots pine (*Pinus sylvestris* L.) has been reduced to a highly fragmented patchwork totalling around 150 km^2. Although these fragments represent only a far-flung isolate of the species' vast continental distribution, in Britain the tree is symbolic of the northern Highlands and a recognizably 'Scottish' landscape. Hence there is considerable motivation, for aesthetic, biological and commercial reasons, to preserve and increase the remaining native fragments. Ecologically, the species crosses a substantial gradient within Scotland, from low-lying, highly oceanic sites in the north-west, to higher, more continental sites in the east (e.g. annual rainfall may vary from >3000 mm to ~700 mm), a distance of no more than 200 km. Although local variation has been recognized for years, a systematic, high-resolution assessment of genetic and phenotypic differentiation has not yet been carried out. In this study we aimed, by integrating studies of phenotypic, molecular-marker and candidate-gene variation, to assess the extent to which the Scottish Scots pine population is structured and resolve whether observed patterns derive from population history or local natural selection. Collections of seed from 21 native Scots pine sites across Scotland were used to establish a progeny trial comprising 3360 seedlings. Over 3 years, all seedlings were assessed for growth and phenology; half were genotyped at 6 microsatellite loci. At the same time, variation at a set of 12 candidate gene loci was assessed in 10 trees per population. Significant differences were observed in total growth and timing of bud burst among sites, although the major proportion of variation tended to be within site. Neutral genetic data suggest there is substantial gene flow among populations; however, there were regional differences in the extent of nucleotide diversity and linkage disequilibrium. In combination, the data suggest that the Scottish population derives from multiple ancestral sources and experienced substantial bottlenecking during colonization; subsequently, populations may have adapted to local conditions, most noticeably at the extremes of the east-west environmental gradient. Contemporary genetic diversity is therefore a product of both history and natural selection; knowledge which will benefit conservation.

Keywords: adaptation, bottleneck, nucleotide diversity, population differentiation

META-ANALYSIS OF WITHIN-POPULATION GENETIC DIVERSITY ACROSS THE MEDITERRANEAN BASIN

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Understanding which ecological processes shape biodiversity is vital to the sustainable management of our global environment. Most biodiversity investigations are done at species level. However, analysing population genetic data in a biogeographic framework can uncover past ecological events that have left a significant imprint on population structure. Seminal studies on Mediterranean forest tree species showed a spatially-structured within-population genetic diversity (GD) in conifers and some angiosperms, with greater diversity in the eastern Mediterranean than in the western Mediterranean. This spatial structure is not congruent with that of species diversity, organized in regional hotspots of endemism and richness. A possible cause for this GD pattern is the climate of the Last Glacial Maximum 21 000 years ago, which was drastically harsher in the western Mediterranean than in the Eastern Mediterranean. Such conditions are likely to have modified available ecological niches, with the advent of small refugia in the west and demographic bottlenecks leading to genetic drift and loss of GD. As trees are key organisms driving the patterns biodiversity of terrestrial organisms we set out to decipher the ecological drivers of microevolution in woody plants and their associated organisms. We report a multispecies and biome-wide survey of the GD based on scientific literature. We gathered data from ca. 200 publications into a database holding around 9000 geolocalized references on ca. 220 plant and animal species. GD was regressed to longitudinal coordinates of sampled populations and globally analysed by meta-analysis procedures. The influence of covariables such as genetic methods used in primary studies as well as several species life-history traits was also investigated. A global positive effect size was found, corresponding to higher GD in the east than in the west, which remained positive and significant after controlling for latitudinal position of the populations (i.e. the effect of migration from refugia towards higher latitudes at the onset of the Holocene). A refined analysis of this pattern on precise taxonomic groups is also presented. Our findings are discussed relative to the hot-spot structure of plant species diversity and in terms of conservation strategies from genes to species in the Mediterranean Basin.

Keywords: genetic diversity, Mediterranean Basin, meta-analysis

PHENOLOGICAL ADAPTIVE RESPONSES OF OAK AND BEECH TO ALTITUDINAL GRADIENTS

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It is widely accepted that genetic diversity and phenotypic plasticity are driving the evolutionary responses of populations and individuals to environmental change. We report on experiments conducted in sessile oak (*Quercus petraea* (Matt.) Liebl.) and beech (*Fagus sylvatica* L.) aimed at assessing the importance of both diversity and plasticity in the response to environmental changes generated by altitudinal gradients. The experiment consisted of set of common garden and reciprocal transplantation experiments in two valleys on the northern side of the Pyrenees Mountains and monitoring of bud burst and leaf coloration *in situ* and in the plantations. We found significant clinal differences in phenology among provenances of both species related to the annual temperature of the provenance's site of origin. However, the two species exhibited opposite genetic clines. While oak populations from lower altitude flushed earlier than those from higher altitude, the opposite pattern was observed in beech. The observed patterns were highly consistent from year to year. Phenotypic plasticity accounted for the *in situ* variation observed. We demonstrated that reaction norms of flushing timing to temperature followed linear clinal trends for both species, with an average change of 5.7 days per degree Celsius increase. Timing of leaf senescence exhibited a parabolic trend for beech and no trend in oak. For both species, the length of the growing season increased to reach maximum values for annual temperature ranging from 10°C to 13°C, depending on the population. Based on the reaction norms, populations tend to occupy suboptimal environments, e.g. populations inhabit climates colder than their optimum temperature. The existence of high population differentiation and large magnitude of plasticity suggest that these populations can respond quite rapidly to temperature changes.

Keywords: adaptation, clines, oaks, phenology, plasticity

REGIONAL ADAPTIVE TRENDS IN ALEPPO PINE (*PINUS HALEPENSIS* MILL.) BASED ON FEMALE REPRODUCTIVE COSTS

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Aleppo pine (*Pinus halepensis* Mill.) is an obligate seeder that is well adapted to recurrent fires due to its precocity and fecundity. However, reproduction in harsh environments is costly and must be done at the expense of other processes such as growth and defence. In Aleppo pine these trade-offs are reflected in short height and thin bark. However, local populations can show different degrees of commitment to this strategy, shaped by different fire regimes, climate gradients or historical colonization. Here we present results from 15-year-old family and progeny trials representative of the species range in the Iberian Peninsula and Balearic Islands. Female reproductive investment was compared with tree size and growth between two dates to assess the trade-off between reproduction and growth. The common garden structure allowed us to calculate the genetic basis of these traits at the species, population and family levels with parameters such as heritability, Q_{ST}, coefficient of additive genetic variance and genetic correlations. Our results show negative genetic correlations (up to -0.30) between reproductive allocation and stem volume over bark, pointing to the existence of a genetically-based cost of reproduction. Moreover, heritabilities are higher in reproductive than in vegetative traits, suggesting that reproductive strategy is more important for local adaptation than growth. This is also shown by the negative phenotypic correlation between reproduction and relative growth rate between measurements and the negligible family variance for this last trait. Therefore, while reproductive strategies in Aleppo pine might be different between genotypes, costs of reproduction are similar. This information will be useful when formulating recommendations to increase the resilience of forest stands by, for example in this case, increasing early reproduction to counteract the effects of climate change.

Keywords: costs of reproduction, *Pinus halepensis*, quantitative genetics, reproductive allocation

DISTRIBUTION OF GENETIC VARIATION RELEVANT TO ADAPTATION TO LOCAL CLIMATE ACROSS DIFFERENT GEOGRAPHIC AND TAXONOMIC SCALES IN *PICEA*

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By characterizing the distribution of genetic variation underlying adaptation to local climate, we hope to develop a more comprehensive understanding of the capacity of natural conifer populations to adapt *in situ* to new climates. Using a population genomics approach, we have identified over 300 single nucleotide polymorphisms (SNPs) in candidate genes for bud set timing and cold hardiness in Sitka spruce (*Picea sitchensis* (Bong.) Carrière). Of these, 35 are significantly associated with and collectively explain a substantial proportion of phenotypic variation in timing of bud set and cold hardiness. To better understand the capacity of populations to rapidly adapt to new climates, we are investigating the distribution of these SNPs at a variety of spatial scales within Sitka spruce, from the extent of local spatial genetic structure within central and peripheral populations to the occurrence of SNPs across the range of Sitka spruce. We are also using these and other SNPs to characterize genetic structure, introgression and local adaptation within two large spruce hybrid zones in British Columbia: the introgression zone between *Picea sitchensis* and *P. glauca* (Moench) Voss (white spruce), associated with the ecotone between the maritime and continental climates in northern British Columbia, and the elevationally stratified hybrid zone between *P. glauca* and *P. engelmannii* Parry ex Engelm. (Engelmann spruce) distributed widely across large, mountainous regions of south-central British Columbia. A total of approximately 2000 individuals sampled from natural populations are currently being genotyped for several hundred SNPs. Patterns of putatively adaptive variation within and among these species at a variety of spatial scales will be presented.

Keywords: bud set, cold hardiness, genetic structure, *Picea*, SNP

POPULATION VARIATION FOR FROST TOLERANCE IN MARITIME PINE (*PINUS PINASTER* AITON.)

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Low temperature is one of the major abiotic stresses limiting plant growth and survival, especially when frosts are not preceded by a slow temperature decrease. It is thus central to local adaptation of plant populations. Maritime pine (*Pinus pinaster* Aiton.) shows high levels of differentiation between provenances in traits related to growth and life history, but so far there is little knowledge on genetic variability for frost tolerance in this species. Frost sensitivity caused major failures in the introduction of maritime pine from the Iberian Peninsula to France in the past century. However, preliminary results with a single provenance from central Spain showed an LT_{50} value of -20ºC for secondary needles in winter, which may indicate genetic variability for frost tolerance among populations of the species. The objective of this work is to obtain a reliable method for evaluating frost tolerance in maritime pine and to determine changes in frost tolerance during the autumn hardening period, winter and spring dehardening. The experiment used 4-year-old saplings from 10 different Iberian provenances, chosen to cover a wide range of thermal environments. The damage caused by frost to leaf tissues was evaluated through artificial freezing and electrolyte leakage, combined with chlorophyll fluorescence measurements prior to the freezing tests to evaluate the general status of plants. F_v/F_m values decreased from November to December following temperature decrease, from an average of 0.83 to 0.70, with no differences among provenances. The artificial freezing tests showed that when plants were exposed to progressively lower night-time temperatures during autumn and winter, LT_{50} declined significantly in all populations and by January all were capable of enduring very severe frosts (LT_{50} ranging from -23ºC to -26ºC). Differences between populations were much lower than expected considering original thermal environment. These results should be confirmed in field experiments, but so far they do not indicate a high frost sensitivity of Iberian populations.

Keywords: acclimatization, artificial freezing tests, electrolyte leakage, local adaptation, *Pinus pinaster*

MULTI-ENVIRONMENT EVALUATION OF *PINUS HALEPENSIS* MILL. AND *PINUS BRUTIA* TEN.: GENETIC VARIABILITY AND ADAPTIVE POTENTIAL IN THE FACE OF CLIMATE CHANGE

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Low-elevation Mediterranean pines of the *halepensis* group (*Pinus halepensis* Mill., *P. brutia* Ten. and *P. eldarica* Medw.) are valuable and prominent forest tree species with wide distributions across the Mediterranean basin. The ability of the species to grow in the adverse climatic conditions of the Mediterranean region, combined with their fast growth in favourable sites and their ability to endure forest fires, render them irreplaceable for Mediterranean ecosystems especially in the face of climate change. Seven *P. brutia* provenances and 12 *P. halepensis* provenances representing the natural distribution of the two species in Greece were tested in two multi-environment trials established across different environmental gradients of the country. The multi-environment testing of *P. brutia* and *P. halepensis* was carried out in five and three different environments, respectively. Height growth, diameter at breast height and survival were recorded in both species at 8 years of age across all trials. The analysis revealed significant G×E interaction for the traits studied in both species. GGE biplots for each species revealed environmental clusters and identified the best-adapted genetic material for each environment. Stability of performance for the traits studied varied among the populations of the two species; the most stable provenances were identified. The results indicate the ample genetic variation harboured within the natural populations of the two species growing in Greece and the adaptive potential of specific populations in adverse environments. As climate change towards greater aridity and higher temperature will challenge natural populations of both species, their plasticity and adaptive potential is explicitly discussed.

Keywords: G×E interaction, GGE biplots, *P. brutia, P. halepensis*, stability of performance

IDENTIFYING ADAPTIVE GENETIC DIVERSITY IN RELATION TO ENVIRONMENTAL VARIATION: PROMISING BOULEVARD, BUT PEPPERED WITH PITFALLS

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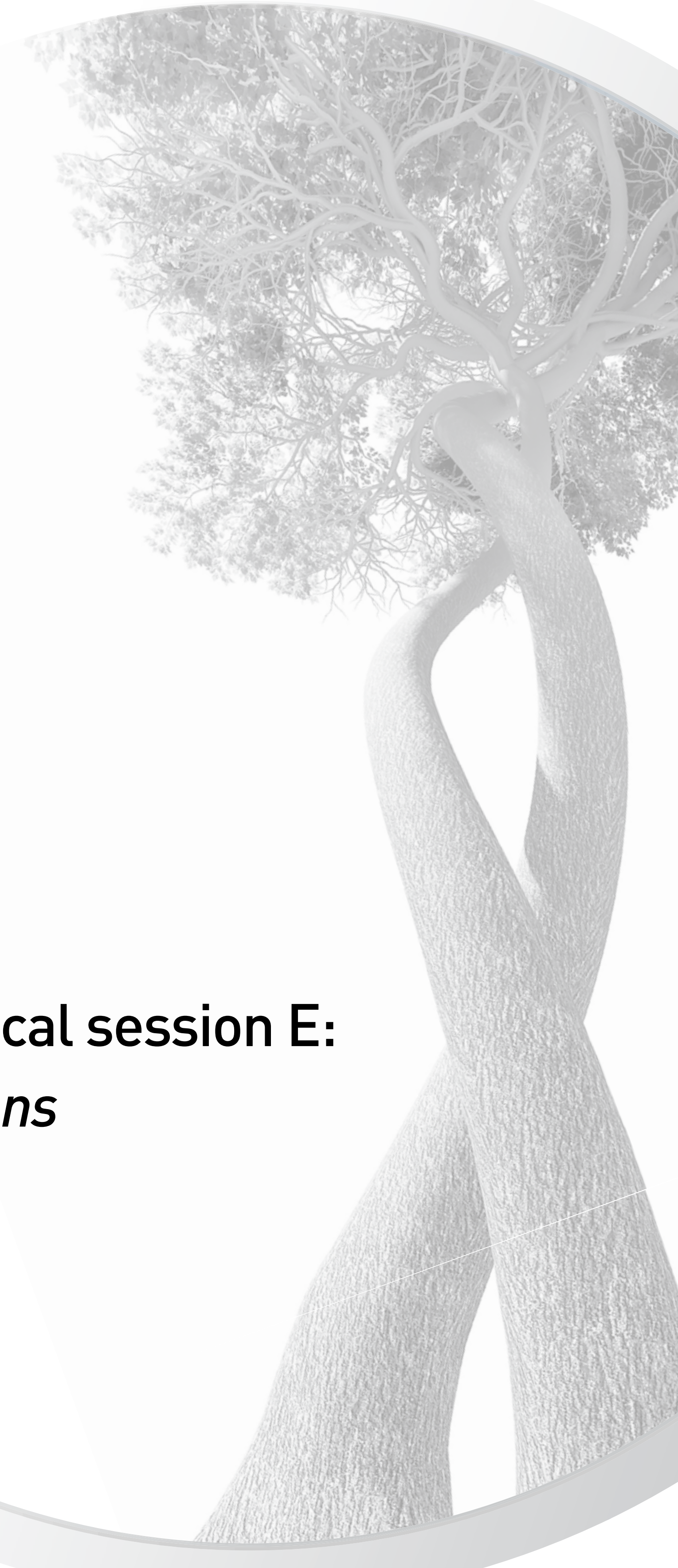
Genetic diversity is a fundamental prerequisite for the adaptive potential of populations. Such adaptive potential is of great importance with regard to changing environmental conditions, but also in light of the conservation of genetic variation as a key component of biodiversity, particularly so in rare species or in small, isolated populations in marginal habitats. For decades, researchers were largely restricted to assessing genetic diversity based on neutral molecular markers, often implicitly presuming that such diversity, e.g. in terms of heterozygosity, may be indicative of adaptive potential or even fitness. However, there is contrasting evidence about such a relationship, which is why attention has recently been directed towards identifying loci of ecological relevance ('outlier loci') and studying nucleotide variation in potentially adaptive genes. We highlight current strategies for studying putatively adaptive genetic diversity in plant populations in relation to environmental variation in a landscape context (landscape genetics). While new genomic tools are becoming available at an unprecedented speed, statistical methods and the theoretical framework of landscape genetics are still lagging behind. We put particular emphasis on the problem of separating adaptive from purely spatial and historical signals, which both may affect allele frequencies in a similar manner. Using data from our own work and the literature, we exemplify currently available methods to account for non-adaptive signals in the search for selective loci, i.e. to identify 'adaptive islands' in the 'sea of genomic neutrality'.

Keywords: adaptation, environmental variation, gene sequences, genetic diversity, genome scan, single nucleotide polymorphisms



Parallel technical session E:

Mitigation options

SEED TRANSFER RECOMMENDATIONS INFERRED FROM PROVENANCE TESTS IN OAKS

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For most forest trees, there is no genetic improvement programme. Seeds are collected in natural stands, which are often selected for their phenotypes. Throughout Europe different regulations limit transfer of seed and breeding material between geographical zones known as provenance regions. These regulations assume that local genetic materials are adapted to local conditions. Climate change has made this strategy obsolete and foresters are seeking new regulations that promote adaptation of their forests to future climate. Previous studies have shown that, globally, Scots pine (*Pinus sylvestris* L.) and lodgepole pine (*P. contorta* Dougl.) populations are adapted to warmer climates than their local climate but there are differences along a north–south gradient. Southern populations are adapted to cooler climate than their local climate whereas northern populations are adapted to warmer climate than their local climate. These results could be applied in different ways: (i) genetic material from the north could be moved south because they have better performances (survival and growth), (ii) local genetic resources could still be used because they have enough plasticity and are adapted to local conditions, or (iii) genetic material from the south could be planted in order to prepare for climate change, but in this case we accept lower performance for the short term. In this talk we will analyse data from French provenance tests of sessile oak (*Quercus petraea*) in the frame of climate change. The provenance test network comprises four experimental plantations where 107 sessile oak populations are tested. We discuss the different possible and contradictory strategies, and propose new ways of managing genetic resources for this species.

Keywords: climate change, genetic resources management, *Quercus petraea*

ENSURING THE SUSTAINABLE REGENERATION OF CALEDONIAN SCOTS PINE (*PINUS SYLVESTRIS* L.) POPULATIONS UNDER A CHANGING CLIMATIC REGIME

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In Scotland, Caledonian Scots pine (*Pinus sylvestris* L.) woods persist in 84 fragments that cover less than 1% of their previous extent. Current guidelines suggest that planting stock should be derived from local seed sources, which are believed to be best adapted to that particular local environment. To this end, seven 'origin zones' have been identified, based on a relatively small interpopulation variation in monoterpenes. This work reappraises the basis for and the suitability of the current origin zones, revisiting the monoterpene basis for the zones, using neutral microsatelite DNA markers, and considering adaptive characters such as growth of seedlings. Using 30 trees from each of 21 sample populations, three from each of the seven origin zones, we analysed needle mono- and sequiterpene markers and developed a set of robust new, selectively neutral, microsatellite DNA markers. Neither our revised monoterpene and sesquiterpene analysis nor microsatellites support the differentiation between the current zones. We collected seed from the 21 populations and conducted a common environment experiment at two contrasting locations, Aberdeen, on the east coast of Scotland (cool, dry climate) and Inverewe, in north-west Scotland (warm, wet climate), to test whether seedlings of different origin zone, population and family (groups of half-sibs) differ from each other with respect to growth characteristics, timing of bud burst and cessation of growth. These adaptive characters are among those that may influence the survival, growth and associated biodiversity of the trees under different climatic regimes. There was significant variation between the seven origin zones in 9 out of 10 growth characteristics, including timing of bud burst in year 2 and stem diameter. Response to contrasting climatic regimes varied with the zone of origin for three characteristics (seedling stem diameter, date of bud burst and number of buds set). These initial results suggest that although the reanalysis of monoterpenes and the new microsatellite markers do not support the current origin zones, growth or other adaptive characteristics may form a suitable basis on which to classify and conserve our populations of Scots pine, predict their response to climate and ensure the long-term persistence of regenerating populations under changing climatic regimes.

Keywords: adaptive characters climate change, *Pinus sylvestris*

SCOTS PINE (*PINUS SYLVESTRIS* L.) GROWTH RESPONSES TO VARIABLE ENVIRONMENTAL CONDITIONS AS SEEN BASED ON IUFRO 1982 EXPERIMENT

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Regression models were used to examine survival, growth and quality traits of European populations of Scots pine (*Pinus sylvestris* L.) included in the IUFRO 1982 Scots pine provenance trial in relation to differences in climate between the original location of the provenance and the trial site. Trees in the trial plots in Poland were measured 25 years after initial planting. Growth data were expressed in standard deviation units in order to compare results from different years. The summarized results from all available experimental plots across Europe showed strong clinal variation in growth and survival traits and a large potential for adaptation to changing climate. Populations from central Europe were fastest growing, while those from northern Europe were characterized by straight stems. The analyses utilized the set of global climate data available at WordClim.org with a spatial resolution of a square kilometer. Regression models showed that growth and survival generally decreased from west to east when a population was transferred from its place of origin to a distinctly different location. Strong relationships were found between volume of trees and transfer distances measured as a difference of annual average air temperature of provenance origin and planting sites. Local populations were not always among the best performing in their home location. Artificial populations established by humans (e.g. at Pornóapáti, Hungary, and Ardennes, Belgium) grew better than natural populations. Results indicate that provenance trials can be efficiently used to predict growth response of particular forest reproductive material transferred in to new environmental conditions.

Keywords: climate, geographical gradients, *Pinus sylvestris*, provenance trials, seed transfer

EVALUATION OF GENETIC RESOURCES OF EUROPEAN BEECH (*FAGUS SYLVATICA* L.) BY INTERNATIONAL PROVENANCE TRIALS

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Sixty field trials (common gardens) in 22 countries throughout the distribution range of European beech (*Fagus sylvatica* L.) were jointly evaluated. Progenies of 200 seed sources (provenances) representing the whole distribution range of European beech have been grown in these field trials since 1995. Survival and height growth vary markedly among the provenances and across sites. Variation among sites is correlated with site quality in terms of location and edaphoclimatic variables. Variation among provenances is complex and cannot be explained simply by current regional adaptation patterns or possible influences of the site in which a provenance is growing in relation to those at its place of origin. At certain sites, some geographically distant provenances are performing unexpectedly well, whereas local provenances frequently are not among the best-performing accessions. The results suggest that a general improvement in growth would result from movement of a provenance north- and westwards by 50 to 100 km, which corresponds to an increase in summer temperature of 0.5°C and an increase in summer precipitation of 100 mm. However, such a shift would not result in growth improvement in all provenances. Accordingly, interactions between genotype and environment are considerable, which complicates provenance recommendations. Time of leaf flushing varies distinctly among provenances and across sites. The trait is closely correlated with amount of winter chilling and increasing temperature sum in spring, with an inherent threshold temperature sum regulating the flushing time depending on the particular late frost risk to which a provenance is adapted. Having been running for 12–15 years the field trials offer manifold possibilities for scientific study of growth expressions in a set of beech seed sources growing simultaneously at numerous locations throughout Europe. The data may also serve to verify predictions about the future distribution range of beech under certain climate scenario assumptions.

Keywords: adaptation, climate change, *Fagus sylvatica*, genotype by environment interaction, provenance trial

ASSISTED MIGRATION TO ADDRESS CLIMATE CHANGE: RECOMMENDATIONS FOR TREMBLING ASPEN (*POPULUS TREMULOIDES* MICHX.) IN WESTERN CANADA

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Bioclimate envelope models are widely used to predict changes to species habitats under projected future climate in an effort to guide species choice for uncertain future climates in reforestation programmes. It is also acknowledged, however, that most wide-ranging tree species should probably not be modelled as a homogenous unit because they consist of different populations that are adapted to the local environments in which they occur. This genetic structure is reflected in forest management through seed transfer guidelines or seed zones, which restrict movement of seed sources to avoid maladaptation. A reforestation strategy for uncertain future climates must therefore go beyond species recommendations and determine which locally adapted genotypes should be used at particular planting sites. We present a modelling approach to address this issue. In a case study on trembling aspen (*Populus tremuloides* Michx.), we subdivide the species range into zones that represent similarly adapted genotypes, and then use regression tree analysis to predict the distribution of habitat for these genotypes under multiple climate change scenarios. Subsequently, we use a consensus approach to determine the genotype that emerges as best adapted under the majority of climate change scenarios. We also report the degree of uncertainty in making a recommendation for locally adapted planting stock. In the case of aspen, recommendations for moving planting stock 1° to 2° latitude north can be made with high confidence over a 10–20-year planning horizon. However, confidence in planting stock recommendations decreases dramatically for the 2050s and 2080s, even for areas where species habitat is projected to be maintained with high certainty. Nevertheless, it appears unlikely that by the 2050s and 2080s aspen planting stock that is adapted to moist environments of the Rocky Mountain Foothill ecosystems and the adjacent Boreal Plains zone of western Canada could still be deployed in the study area.

Keywords: adaptation, assisted migration, bioclimate envelope modelling, climate change, trembling aspen

DIFFERENTIATION AND SPATIAL GENETIC STRUCTURES IN DIFFERENTLY MANAGED ADULT EUROPEAN BEECH (*FAGUS SYLVATICA* L.) STANDS AND CORRESPONDING JUVENILE TREES

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Genetic variation patterns were observed at five microsatellite gene loci in stands of European beech (*Fagus sylvatica* L.) from the central (Solling, Germany) and eastern (Zemplén Mountains, Hungary) parts of the tree's distribution range. Regional genetic differences were analysed as well as the genetic consequences of two frequently applied management systems (shelterwood and small canopy openings) on adults and juvenile plants. Some marked genetic differences between the two regions were found, which could be explained by differences in recolonization history. The two silvicultural methods also had distinct impacts on the genetic composition of the offspring generation, with similar trends in both regions. For single canopy openings, the differences between adult and juvenile trees were markedly greater compared with trees under shelterwood management. However, pooling juvenile trees from several gaps substantially decreased these differences. This indicates that genetic differentiation among juvenile trees from the openings were largely due to genetic differences among adult trees surrounding the openings. The analysis of spatial genetic structures revealed different characteristics between shelterwood cut and canopy-opening systems in both regions and generations. Low densities of adult trees in shelterwood plots resulted in weak spatial genetic structures among adult trees. However, their offspring exhibit clear family structures. The small canopy openings show spatial genetic structures with considerable family characteristics for both the parental and the offspring generation. The offspring from canopy openings present a sharper spatial separation between different families than do those from shelterwood plots. These results point towards specific impacts of silvicultural management practices on genetic structures effective at small spatial scales in addition to the ecological advantages of canopy openings over continuous-cover forestry practices.

Keywords: *Fagus sylvatica*, genetic differentiation, management systems, microsatellite loci, spatial genetic structure

ESTIMATING EFFECTIVE NUMBER OF MOTHER TREES UTILIZED FOR POPULATION-WIDE SEED COLLECTIONS IN SCOTS PINE (*PINUS SYLVESTRIS* L.)

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Maintaining high genetic diversity of forest tree populations may increase their ability to persist under climate change. Thus, seed for reforestation or *ex situ* conservation purposes should be collected from a large number of mother trees. However, other than on-site inspections at the time of seed harvesting, there is no simple way to check the number of trees from which seed is collected. Here we present a novel method for estimating the effective number of trees from which seeds were collected that can be applied to conifers. In conifers, seed consists of a megagametophyte, which has a chloroplast genome identical to its mother tree, and an embryo with a chloroplast genome derived from the paternal tree. Assuming random mating, it is possible to demonstrate that difference in chloroplast polymorphisms observed between paternal and maternal pools of a seed sample is a function of the effective number of female parents. Such analyses are especially feasible in Scots pine (*Pinus sylvestris* L.), which has a high diversity of chloroplast haplotypes identified using chloroplast microsatellites (cpSSR). The precision and statistical properties of this method were investigated based on the polymorphism of 10 cpSSRs in a broad sample of 200 Scots pine trees. Simulations indicated strong robustness of the estimation procedure, especially when the actual numbers of mother trees were low. The precision of the estimates increased as the seed sample increased. However, in the case of Scots pine a sample size of 100 seeds seems to be sufficient for achieving precise estimates of the effective number of mother trees. The method was then used to estimate the effective numbers of mother trees of three randomly chosen seed collections. Using 100 seeds per sample, we found that the effective number of mother trees ranged from 7.5 to 28.9. Such low effective numbers of mother trees may result from the restricted number of trees from which seeds were collected or from highly uneven collection of seeds from a relatively large number of trees. The seed collection exhibiting the lowest effective number of mother trees may be of questionable value for reforestation or *ex situ* conservation.

Keywords: chloroplast microsatellites, effective number of mother trees, *Pinus sylvestris*, seed collection

Stakeholders’ event

THE CONTRIBUTION OF GENETICS AND GENOMICS TO THE CONSERVATION OF TREE GENETIC RESOURCES

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The recent development of new high-throughput sequencing and genotyping techniques has revolutionized the way we now do population and evolutionary genetics, with application to the conservation and sustainable management of forest genetic resources. Nowadays, even for non-model species, hundreds if not thousands of genes are easily sequenced, providing new tools to evaluate adaptive variation at the molecular level and to study demographical processes with a resolution that was unattainable just a few years ago. However, identification of the relevant genes is still a daunting challenge, as many forest tree species have large, complex genomes (for instance, those of conifers), and robust inferences rely on large sample sizes and association genetic studies based on multisite common garden tests, which are difficult to establish and maintain. In this talk, we review recent progress in this topic and identify active fields of research for the coming years. We also describe the main uses of these new genetic and genomic resources for the conservation and management of forest tree genetic resources, including the identification and definition of conservation genetic units, the monitoring of changes in genetic diversity due to environmental or human-induced pressure and the development of tools for tree origin certification and 'assisted migration'. Finally, we present a cautionary tale about the use and misuse of genomic tools in sustainable forest management. Although our case studies focus on Mediterranean species, the conclusions drawn are far reaching and can be directly applied to other forest trees.

Keywords: conservation, forest trees, genomics, genetic resources, high-throughput genotyping, management

DNA FINGERPRINTS TO DETERMINE THE ORIGIN OF FOREST REPRODUCTIVE MATERIAL: A CASE STUDY IN SESSILE OAK (*QUERCUS ROBUR* L.)

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Collection and trade of reproductive material of forest species is regulated in many countries by certification schemes because well-adapted, high-quality reproductive material is key to success of forest plantations. To comply with these regulations and to promote the use of reproductive material of known origin, efficient tools for the identification of the origin of reproductive material are needed. Existing methods require complex and expensive storage of reference samples at each step in the chain-of-custody of forest reproductive material. We investigated the feasibility of a new approach using DNA fingerprints. This would require only a sample of DNA of adult trees within seed stands. Suspect plants or groups of suspect plants would be checked by use of multilocus genotype assignment. Initially, we simulated the impact of several factors on the accuracy of a correct assignment of individuals or groups of individuals to their source populations using a baseline population of sessile oak in a 50 km × 50 km landscape. The group-wise assignment proved to be robust against low sample sizes, missing data and errors in genotyping. When group samples included at least four individuals and eight loci, the proportion of correctly assigned groups was greater than 93%. Subsequently, we made a field test with samples of adult trees and progenies from 10 registered seed stands of *Q. robur* in western Germany. Nine highly variable nuclear microsatellites were used to genotype each individual. In all cases the progenies (groups of individuals) were correctly assigned to the adult population with a score > 0.95. The conclusion of the field study and the simulations is that wide application of this new approach to checking the origin of forest reproductive material is feasible.

Keywords: forest reproductive material, enotype assignment, origin control, regulation, simulation

Plenary session – Day 3

A COMMUNITY AND ECOSYSTEM GENETICS APPROACH TO CLIMATE CHANGE

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Climate change is one of the most important ecological events of our time. It is also likely to be a major evolutionary event. An important approach for understanding both the ecological and evolutionary impacts of climate change on the ecosystems of the world is to first quantify how climate change is an agent of selection that alters the distribution and genetic structure of foundation plant species. The genetic structure of foundation species is especially important to quantify as these species are, by definition, community and ecosystem drivers. Thus, whatever affects their distribution and genetic structure also will have cascading effects on the rest of their respective ecosystems. Here, I develop specific examples showing that: 1) climate change is an agent of selection on foundation species; 2) changes in the genetic structure of foundation species alter community richness, abundance, composition and biodiversity; 3) changes in the genetic structure of foundation species affect diverse community members including mycorrhizal mutualists of plants, nurse plant associations and arthropods occupying multiple trophic levels; and 4) changes in the genetic structure of foundation species alter the evolution of dependent community members. Our findings and those of others indicate that climate change has the potential to fundamentally change the evolutionary trajectories of whole communities and ecosystems. I propose an experimental approach involving provenance trials of interacting foundation species to provide the data to develop a management plan that would lessen the negative impacts of climate change and preserve the greatest biodiversity in the face of what could become a major extinction event.

Keywords: biodiversity, climate change, community genetics

WHAT CAN WE LEARN ABOUT EVOLUTIONARY RESPONSES TO ENVIRONMENTAL CHANGE FROM ECOLOGICAL POPULATION GENOMICS?

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Adaptive genetic variation in forest tree populations is the most important material for adaptive evolution and speciation in forest tree species. High-throughput SNP genotyping and massively parallel sequencing technologies and other modern methods of evolutionary, ecological and population genomics allow researchers now to combine genome-wide sequence analysis with population sampling and to correlate individual genotypes with environmental factors and ecological gradients. These technologies and methods are briefly reviewed and an integrated ecological population genomics approach to understanding the molecular basis of population adaptive divergence and evolution of adaptive traits is offered. Alleles (haplotypes) at candidate genes for adaptive traits and their effects on phenotypes can be efficiently characterized via sequencing and association mapping. Gene target enrichment methods and cDNA profiling, in combination with high-throughput sequencing and genotyping, can provide invaluable information on nucleotide variation and detailed transcriptional profiles, respectively. Nucleotide variation can also be used to test neutrality under different molecular evolution and demographic models and to find loci under selection. Allele frequencies of multiple genes can be estimated in native populations to identify patterns of adaptive variation across heterogeneous environments. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), loblolly pine (*Pinus taeda* L.) and other southern pine (*Pinus* subsection *Australes*) comparative studies are used as case studies to demonstrate comparative ecological and population genomics methods. Ecological population genomics, through deciphering allelic effects on phenotypes of multiple genes and identifying patterns of adaptive variation at the population level, represents now a useful and cost-effective tool to study adaptation and evolution in forest trees.

Keywords: adaptation, ecological population genomics, high-throughput SNP genotyping, massively parallel sequencing, pines

WHY GEOGRAPHIC RANGE LIMITS? ECOLOGY, EVOLUTION AND CONSERVATION

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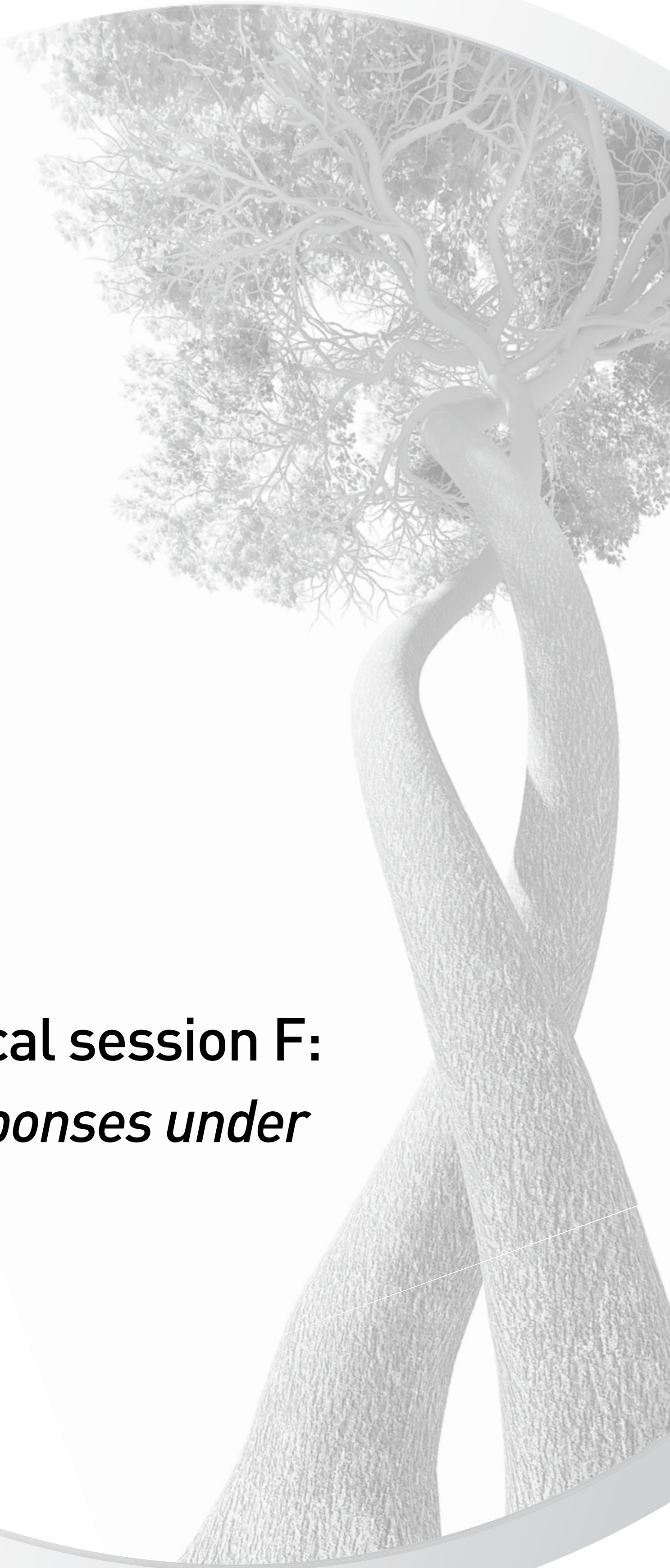
Virtually all organisms have limited geographic distributions. Yet despite long-standing interest in how and why range limits occur, we still have a poor understanding of how ecological factors limit geographic distributions in the short term and why natural selection does not cause populations at range limits to adapt to extreme conditions and spread outward over evolutionary time. The hows and whys of range limits are particularly relevant for management strategies aimed at conserving species and communities in rapidly changing environments. My students and I have used a combination of large-scale geographic surveys, population-genetic analysis, common garden experiments and reciprocal transplants to test theoretical explanations for range limits. Much of our work uses plants endemic to Pacific coastal dunes because these species exhibit very simple, near-linear, one-dimensional distributions. Our results to date challenge some of the most widely held hypotheses concerning the demographic structure of geographic ranges and the ecological and genetic factors that prevent range expansion. We have also studied invasive species to investigate the formation of geographic ranges over contemporary time scales, and have found that ecological and evolutionary processes may interact to determine the extent of range expansion.

Keywords: ecological processes, evolution, geographic range



Parallel technical session F:

Community responses under climate change

SYMBIONT INVENTIONS: GENOMES OF THE BASIDIOMYCETE *LACCARIA BICOLOR* AND THE ASCOMYCETE *TUBER MELANOSPORUM* REVEAL EVOLUTIONARY INSIGHTS INTO MYCORRHIZAL SYMBIOSIS

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Plants gained their ancestral toehold on dry land with considerable help from their mycorrhizal fungal symbionts. This kind of symbiosis contributes to the delicate ecological balance in healthy forests. The genomic sequences of two representative symbiotic fungi, *Laccaria bicolor* (Maire) P.D. Orton and *Tuber melanosporum* Vittad., have been released. At 60 and 120 Mbp, the genomes of *L. bicolor* and *T. melanosporum* are bigger than previously sequenced fungal genomes. The size is partly explained by the large number of transposable elements, which constitute 22% and 65% of the genome of *L. bicolor* and *T. melanosporum*, respectively. A striking expansion of several multigene families occurred in *L. bicolor*, suggesting that adaptation to symbiosis was proceeded by gene duplication. In contrast, the evolution toward symbiosis in *T. melanosporum* led to the loss of gene families. These symbionts lack enzymes involved in degradation of carbohydrate polymers of plant cell walls but maintain the ability to degrade other soil polymers, pointing towards the dual life that mycorrhizal fungi possess, that being the ability to grow in soil using decaying organic matter while serving as a custodian of living plant roots. In this presentation I discuss how genomics and genome-wide transcriptomics allowed us to identify genes of central importance in controlling the steps of formation of ectomycorrhizae. In *L. bicolor*, the most highly expressed secreted protein, MiSSP7, accumulates in hyphae colonizing its host's apoplast and is targeted at the poplar nucleus, where it alters gene expression. RNAi-inactivation of the gene coding for MiSSP7 showed that this gene has a decisive role in the establishment of the symbiosis. The unravelling of these secretomes provides tantalizing hints about differences between symbiotic fungi and their saprotrophic and pathogenic relatives.

Keywords: mycorrhizal symbiosis, *Laccaria bicolor*, *Tuber melanosporum*, genomics, genome-wide transcriptomics

THE IMPACT OF GEOGRAPHIC VARIATION IN THE KEYSTONE TREE SPECIES, PEDUNCULATE OAK (*QUERCUS ROBUR* L.) ON SUSCEPTIBILITY TO INSECT HERBIVORY: A TRANSLOCATION EXPERIMENT

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The strength of biotic interactions between trees and the organisms that depend upon them is a key factor in determining the consequences for biodiversity of anthropogenic or natural changes in forest populations. Tree migration in response to environmental changes, by natural gene flow mechanisms or by deliberate movement of germplasm such as that proposed under the ‘predictive provenancing’ strategy for climate change adaptation, may have far reaching consequences for biodiversity where local populations of trees and their associates have become co-adapted. Research efforts are now underway to measure these interactions at a range of geographic, phylogenetic and temporal scales and in various biological groups. To assess the extent to which geographical variation in plant populations interacts with the native insect herbivore community, a reciprocal transplant experiment was designed, using the keystone tree species, pedunculate oak (*Quercus robur* L.). The experiment incorporated multiple open-pollinated families sampled from oak forests in each of three sites in Hungary, France and Scotland. The experiment was established as a randomized block design with two replicates of 15 single-provenance, 12-seedling plots, giving a total of 360 seedlings at each experimental site. Plots comprised 1, 2 or 4 families, incorporating a genetic diversity gradient within each provenance. For each seedling, phenology and growth were monitored, and insect herbivory rates were recorded in detail at three time points during the summer of 2009. Here, we present results from first year assessments of the trial, with a focus on the extent to which provenance translocation affects tree susceptibility to insect herbivores.

Keywords: community, genetic diversity, herbivory, insect, provenance, *Quercus robur*

HOST–INSECT INTERACTIONS: CANDIDATE GENES IN OAKS AND INSECTS CORRELATED WITH DEFENCE RESPONSE, DETOXIFICATION AND PHENOLOGY

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Interactions between trees and their pathogens/parasites are a driving factor for developmental and therefore also for evolutionary processes in forest ecosystems. Three major traits can be identified as determinants in the evolution of plant–insect interactions and adaptation of insects to their environment. These are two traits of insects—host use and phenology—and the defence response of hosts against feeding insects. We observed fundamental differences in the defoliation level of individual pedunculate oak (*Quercus robur* L.) trees in Germany. Some of the trees seem to be tolerant of the insect grubs (T oaks) while others seem to be conspicuously susceptible (S oaks). Our interpretation of this observation is that oaks generally are sensitive to insect feeding, but individual trees may be less susceptible or more tolerant. We grafted some of these T and S oaks and used them for several feeding experiments with tortrix (*Tortrix viridana* L.) larvae. The preference of larvae for feeding on the S oaks was significant. In our ongoing research we are aiming to identify candidate genes correlated with (a) the defence response after insect feeding in oaks, (b) the defence in insects after feeding on either tolerant or susceptible oaks (detoxification), and (c) the adaptation of insects to different phenology in their hosts. For this purpose we isolated the RNA from T and S oaks after they had been fed on by *T. viridana* larvae and the RNA from insect guts in the same treatments. We could identify some candidate genes in *T. viridana* correlated with detoxification using cDNA-AFLP and sequencing of differentially expressed fragments. We sequenced mRNA from T and S oaks after they had been fed on by *T. viridana* to identify differences in the expression patterns. We identified some hundred differentially expressed fragments. BLASTing for identification of the fragments is still in process. For the identification of candidate genes in *T. viridana* correlated with phenology, we used nine potential phenology genes already known from the literature and BLASTed them against an EST library of *T. viridana* created within EVOLTREE. We designed 28 primers. So far we have been able to reproducibly amplify some fragments in the gene *DH-PBAN*. A further step will be to analyse insect and oak material from different geographic areas in Europe to detect differences in sequences of these candidate genes.

Keywords: candidate genes, host–insect interaction, *Quercus robur, Tortrix viridana*

TESTING THE COMMUNITY GENETICS HYPOTHESIS: EXPERIMENTAL EVIDENCE OF RESPONSE OF INSECT HERBIVORY TO OAK GENOTYPE DIVERSITY

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The community genetics concept suggests that the genetic diversity of keystone species is a main driver of associated species assemblages and ecosystem processes. We tested this hypothesis using insect herbivory on oak seedlings. We planted plots of increasing genetic diversity with all possible combinations of 1, 2, 3 and 4 half-sib families (hereafter genotypes) of *Quercus robur* L. while keeping seedling density and environmental variables as constant as possible. In this way, causal relationships between genetic diversity and insect herbivory could be derived. Overall we observed significantly lower insect herbivory damage in mixtures of oak genotypes that in monocultures, thus supporting the associational resistance hypothesis. However the difference in leaf consumption between pure and mixed plots tended to decrease with increasing number of genotypes, with a larger decrease in damage in two-genotype mixtures than in four-genotype mixtures. This pattern suggests a curvilinear response of insect herbivory to increasing genotypic diversity in oak seedlings. The results are interpreted according to genotype trait dissimilarity and insect herbivore feeding specialization.

Keywords: community genetics, insect herbivory, *Quercus robur*

POPLAR COMMUNITY GENETICS: IDENTIFYING GENOMIC REGIONS ASSOCIATED WITH INSECT AND RUST DAMAGE IN A SEGREGATING *POPULUS* FAMILY

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Forest trees are often keystone species, associated with large numbers of plant, animal and fungal species. Genetic variation in leaf physical and chemical properties of forest trees is thought to influence the extent of these community interactions, e.g. by influencing palatability to herbivores. In order to identify genomic regions associated with susceptibility to insect and rust damage, parents and progeny of a mapping population of poplars (*Populus trichocarpa* × *P. deltoids*, POP1) were assessed for seven types of leaf damage, namely that due to chewers, skeletonizers, leaf miners, leaf rollers and sap suckers, leaf galls and that caused by rust infection. Trees were scored in early (June) and late (August) summer. Greater levels of damage were observed later in the season. Damage levels were highly variable between progeny trees. Damage levels were treated as quantitative traits in a QTL mapping approach. From the June data, one QTL was identified for skeletonizers and one for leaf miners. From the August data, two to four QTLs were identified for each damage type, except for galls and leaf rollers, for which no QTLs were identified. Different QTLs were identified for skeletonizers and leaf miners from the June and August data, indicating potential facultative defence mechanisms being induced later in the season. Alternatively, the causal insect species may have been different between these two time points. For the August data, QTLs for some insect damage types (chewing and leaf mining) co-located in a single position, indicating this genomic region may contain genes related to damage or defence. A single QTL explained between 2.9% and 9.2% of the variance in the trait assessed. The limited co-location between QTLs for different damage types indicates the insect community may respond to genetic variation underlying different polygenic traits depending on the type of herbivory and time of year. These QTLs will be the basis of a bioinformatics study to identify potential chemical mechanisms underlying the variation in insect and rust damage in this hybrid population.

Keywords: hybrid, quantitative trait loci, insects, leaf damage, *Populus trichocarpa*, *Populus deltoides*, quantitative trait loci, rust

THE EFFECT OF LOCAL ENVIRONMENTAL CONDITIONS ON ASSOCIATED ORGANISMS OF LOMBARDY POPLAR (*POPULUS NIGRA* CV 'ITALICA')

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Lombardy poplar (*Populus nigra* cv 'Italica') has been planted all over Europe, mostly as single trees or in rows along roads or around squares. This clone represents a unique opportunity to study local effects (e.g. associated community diversity) on a single genotype. We investigated the effect of local environmental conditions (biogeographical species pools, site conditions, surrounding vegetation) and clines in environmental conditions (climate, latitude) on the diversity of associated organisms, notably insect species. Pairs of trees were selected on at least five sampling sites (mixture of rural and urban) in each of the following countries: France, Germany, Hungary, Italy and Poland. Fifty leaves per tree were selected randomly once a month from May until September and evaluated for damage by six functional groups of insect: chewers, skeletonizers, leaf miners, sucking and sap feedings, gall makers and leaf rollers. Insect species were also determined for leaf miners and gall makers. There was no significant difference in damage levels between pairs of trees at each site, but significant differences were found among sites and among countries for the means of leaves damaged by each functional group. The same leaf miner species were found in the five counties but at different frequencies. The dominant gall-maker species was the aphid, *Pemphigus spyrothecae*, in all countries; however both species composition and dominance varied strongly among sites. Remarkably, species diversity (Shannon-Wiener Index) of gall makers and leaf miners was higher on urban sites than on rural sites.

Keywords: insect associates, genotype, local environment, Lombardy poplar, *Populus nigra*

GENETIC DIVERSITY OF ECTOMYCORRHIZAL SYMBIONTS OF LOMBARDY POPLAR (*POPULUS NIGRA* L. CV 'ITALICA') GROWN IN A RANGE OF ENVIRONMENTS

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Populus is one of the few tree genera with both ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) associations. Little is known about the factors influencing the composition of mycorrhizal fungal communities associated with poplar trees. The frequent interspecific hybridization of poplars complicates field studies aimed at distinguishing the effects of genetic and environmental factors on mycorrhizal communities. Lombardy poplar (*P. nigra* L. cv 'Italica') is a male clone selected in Lombardy, northern Italy, in the 17th century. Spread by cuttings throughout Europe and the USA it has been cultivated as a street and landscape tree in a wide variety of environments. The genetic similarity of Lombardy poplar trees provides an opportunity to determine the effects of local environmental conditions on the composition of mycorrhizal fungal communities associated with a single tree genotype. We examined mycorrhizae of mature Lombardy poplar trees grown under various soil conditions and subject to various types and levels of anthropogenic pressure in Poland. Fine roots were sampled randomly from the upper soil layer in spring and late summer. ECM and AM colonization of the poplar roots were evaluated using the intersection method after cleaning and staining of the root samples with trypan blue. Ectomycorrhizae were sorted into morphotypes based on their morphological characters. DNA was extracted from individual ectomycorrhizae and the rDNA ITS region of the fungal symbiont was amplified by PCR, cloned and sequenced. Molecular taxonomical identification was accomplished using BLAST analysis against sequences in databases followed by phylogenetic analysis (neighbour joining method) of ITS sequences. Alignment with ITS sequences in various databases showed various species of *Tuber*, *Tomentella* and *Hebeloma*, one species each of *Laccaria*, *Helvella*, *Paxillus* and *Thelephora* and possibly several other species as well; we obtained a number of sequences with close matches in the EMBL Genbank to 'uncultivated fungal species' only. The proportion of ECM and AM colonization and the richness and composition of ECM mycobionts associated with the poplar roots differed between study sites, suggesting an effect of local environmental conditions on mycorrhizal symbiosis.

Keywords: anthropogenic stress, arbuscular mycorrhiza, ectomycorrhiza, 'Italica', Lombardy poplar, *Populus nigra*

RELATIONSHIP BETWEEN HOST-GENOTYPE DIVERSITY AND ECTOMYCORRHIZAL COMMUNITY STRUCTURE IN A 6-MONTH-OLD BLACK POPULAR (*POPULUS NIGRA* L.) COMMON GARDEN EXPERIMENT

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Black poplar (*Populus nigra* L.) has been important element of the natural landscape of Europe for several centuries. The development of agriculture and industry, the regulation of river systems and environmental pollution significantly decreased the range of natural stands of poplars and their genotypes. Determination of the genetic diversity of black poplar and developing an understanding the interrelationship between tree genotype and associated groups of organisms (fungi, insects) are important to the protection of natural stands and restoration of this species in Europe. The objective of this study was to analyse the impact of host genotype on ectomycorrhizal colonization of local clones of black poplar. In June 2009 root samples were collected from a field experiment, established in Kórnik (Poland) as a part of ACTION 1 activity (EVOLTREE COMMUNI-TREE group). The experiment consisted of 720 trees of 8 local clones planted in 4 replicated blocks. Each block was divided into plots consisting of six trees. Plots varied in their clonal composition from one to six clones per plot. In this study we present the results of mycorrhizal community analyses on four selected clones (Nos 3, 4, 6 and 9) from the Polish site. Analyses of root samples revealed well developed ectomycorrhizae (ECM), which colonized about 50% of root tips. Significantly more ectomycorrhizal species were observed in non-homogenous (3-, 4-, 6-clone plots) than in single-clone plots. The species composition of ECM fungi differed among the black poplar clones. The most abundant mycorrhizae belonged to *Tomentella* sp. and *Inocybe* sp. The results suggest a relationship between genotype of poplar clones and ECM species diversity.

Keywords: community structure, ectomycorrhiza, genotype, *Populus nigra*

REGENERATION OF ECTOMYCORRHIZAL FUNGI AFTER FOREST FIRE

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Mean annual temperatures are predicted to increase dramatically over the next hundred years. In dry inner-alpine valleys, higher temperatures in summer will result in higher frequencies of drought periods during the growing season. As a consequence, forest fires are likely to occur more frequently, especially in forests on shallow soils on south-facing slopes that are often dominated by Scots pine (*Pinus sylvestris* L.). Recruitment failure of Scots pine after forest fires has often been reported, and we suggest that this is in part due to the impact of fire on ectomycorrhizae associated with Scots pine. Successful seedling growth depends on ectomycorrhizal fungi (EMF) that improve water and nutrient uptake of their hosts. EMF may, however, be destroyed by stand-replacing fires, and we hypothesize that the diversity of EMF is lower on recent forest fire sites than on older sites. We assessed the EMF community composition on a chronosequence of 12 forest fire sites dating from 1990 to 2006 located in Valais, Switzerland, and Aosta, Italy. We took soil samples along transects both in burned areas as well as adjacent undisturbed forest stands and performed a bioassay using Scots pine as an experimental host plant. Subsequently, EMF species were identified by a combination of morphotyping and sequencing of the ITS region. Mean number of species was significantly lower in samples from burned areas than in those from adjacent undisturbed Scots pine stands, indicating a simplification of EMF communities due to fire. To assess the degree of regeneration over time, we compared EMF communities in samples of burned areas with those in samples from adjacent undisturbed forest by calculating the Jaccard similarity coefficient and plotting it against time since burn. Regression analysis showed a slight but significant increase in similarity between community pairs from 45% to 75% within 20 years of a stand-replacing fire, indicating resilience of EMF communities within that time frame. In conclusion, failure in Scots pine recruitment after fire may be to some extent explained by poor EMF diversity soon after the fire. Further studies should investigate the effect of changes in EMF community composition on host-tree performance.

Keywords: ectomycorrhizal fungi, fire disturbance, *Pinus sylvestris*, tree regeneration

Parallel technical session G: *Evolutionary responses under climate change*

THE POPULATION GENOMICS OF ADAPTATION TO PHOTOPERIOD IN EUROPEAN ASPEN (*POPULUS TREMULA* L.)

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The initiation of growth and dormancy represent critical ecological and evolutionary trade-offs in perennial plants. Latitudinal clines in important phenological traits are common in many plants. In European aspen (*Populus tremula* L.) the most important environmental cue regulating dormancy initiation is a shortening of the photoperiod. QTL mapping has implicated genes in the photoperiodic pathway in the control of growth cessation. Here we present data from a study on the genetic basis of variation in phenology in European aspen across a latitudinal gradient. We show that genetic differentiation at neutral markers is low despite strong differentiation in phenological traits, demonstrating local adaptation to the photoperiodic regime. Patterns of nucleotide polymorphism in several genes from the photoperiodic pathway suggest the action of natural selection along the environmental gradient. We also identified SNPs from these genes that we used to test for associations with naturally occurring variation in bud phenology and senescence.

Keywords: dormancy, latitudinal cline, phenology, photoperiod, *Populus tremula*

MODELLING THE IMPACT OF ENVIRONMENTAL AND DEMOGRAPHIC CHANGES ON TREE EVOLUTION

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Theoretical models dealing with local adaptation generally use individual fitness as a parameter driving the process of adaptation. This parameter is either directly determined by the genotype, or indirectly derived from life history traits linked to the genotype. Despite benefits of such assumptions and valuable theoretical concepts that emerged, evolutionary drivers must be partitioned into genotype-phenotype-demography maps to account for complex interactions among genes or traits regarding selection when environment or demography is unstable. In such demo-genetic models, individual fitness is determined dynamically from demographic processes and environment quality, which can vary in space or in time. Trees can be considered model organisms for testing such a theoretical framework, because tree populations usually experience high spatial and temporal heterogeneity due to high longevity, size and dispersal abilities. In order to study short-term evolution in a non-equilibrium population, we used a demo-genetic model on the CAPSIS platform to model Atlas cedar (*Cedrus atlantica* (Endl.) G. Manetti ex Carrière) colonization in south-east France over three generations. We studied interactions between gene flow and environmental spatial heterogeneity during the colonization process by simulating evolutionary trajectories over three generations in two different landscapes (random or gradient variation of a site index) and under high and low levels of seed and pollen dispersal. Three generations were enough for natural selection to lead to genetic and phenotypic differentiation among micro-environments. The positive effect of low gene flow on the maintenance of diversity was more pronounced under the random environment than under gradient variation due to greater differences among sites. Gene flow interacted with spatial pattern of environmental heterogeneity. The divergence between differentiation patterns observed on neutral markers, QTL or adaptive traits increased when environmental conditions were spatially aggregated, more so when seed and pollen flow were low. Thus, selection is more intense along a gradient than in a random environment. Heritability and evolvability decreased differently across generations. Heritability decreases continuously whatever the gene flow or the type of environment, while evolvability decreased continuously when gene flow was low but declined strongly at first and stabilized after the first generation when gene flow was high.

Keywords: *Cedrus atlantica*, demography, genotype, model, phenotype

AN EPIGENETIC MEMORY AFFECTS CLIMATIC ADAPTATION IN NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.)

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We have recently found that Norway spruce (*Picea abies* (L.) H. Karst.) can rapidly adjust its adaptive performance, probably through an epigenetic mechanism. This appears to employ a kind of long-term memory of temperature sum and (probably) photoperiod from the time of its embryo development. In our research we made identical controlled crosses and produced seed lots under controlled temperature and day-length conditions and later observed phenology, growth and hardiness traits in the progenies. It was repeatedly found that temperature conditions during seed set, in particular, influence the phenotypes of the offspring; seedlings from seeds produced under warm conditions have later terminal bud set and reduced autumn frost hardiness than those from seed produced under colder conditions, and thus perform like a more southern provenance. When embryonic clones were derived from mature zygotic embryos and were cultured at different temperatures, the plants cultured under warm *in vitro* temperature were the last to set bud and grew taller than those cultured at lower temperatures. Progenies produced in Norway by central European mother trees had a bud set curve skewed towards that of the local Norwegian performance. A comparison of the performance of seedlings from seeds collected in the same provenance regions in 1970 and 2006 shows that the more recent seed lots consistently produce taller seedlings with a later bud set, probably due to higher temperatures during seed production in 2006. The effect of reproductive environment has been shown to persist for years. It mimics the variation between provenances from different latitudes and altitudes and may explain much of the observed variability in bud set and early height growth between natural populations of Norway spruce. The observed phenomenon suggests an epigenetic mechanism in the developing embryo, either zygotic or somatic, that senses environmental signals such as temperature and influences adaptive traits. Research is underway to understand the molecular basis of this mechanism. We will discuss the implications of this epigenetic phenomenon for the interpretation of provenance differences, for tree breeding and for its possible role in adaptation to climate change.

Keywords: climatic adaptation, epigenetics, *Picea abies*, reproductive environment

PATTERNS OF GENE FLOW IN WHITE OAK STANDS ACROSS EUROPE

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Gene flow, occurring in plants by means of dispersal of pollen and seed, affects the genetic structure of populations, creates opportunities for hybridization and thereby influences the ability of a species to adapt to future environmental changes. The evolution of populations can be studied by comparing different spatial locations, characterized by different climates. Our objective was to improve our understanding of the dispersal function of species and its evolutionary abilities. In order to do so, we measured the extent of gene flow in nine oak stands located in nine European countries. Studying a wide geographical range of stands under very different environmental conditions allowed the evolutionary responses of species to environmental change to be assessed, using space as a proxy for time. Exhaustive sampling of adult trees (mostly sessile [*Quercus petraea* (Matt.) Liebl.] and pedunculate oaks [*Q. robur* L.]) was conducted in geographically continuous plots. The spatial location of each tree was recorded, and acorns were sampled on several mother trees within each stand. Seven of the nine stands were also sampled for seedlings. Genotypes of adults, seeds and seedlings were obtained for four to eight nuclear microsatellite loci. When possible, fathers/parent(s) were assigned to the seeds/seedlings using likelihood ratio calculations. We estimated the mating success of the various male and female parents and constructed pollen and seed dispersal curves for each stand. We compared the trends across stands, and attempted to identify generalities and outliers. Although differences among stands (shape, sampling) clearly affected the results and precluded general conclusions, some common trends could still be detected. Reproductive success distributions were all L shaped, with a few parents generating many offspring and many parents contributing to few offspring. Dispersal curves were better estimated for pollen than for seeds (because more data were available for pollen); rather large confidence bands were obtained, with larger mean distances for pollen than for seeds.

Keywords: dispersal, nuclear microsatellites, oak, parentage, paternity

GENETIC PROCESSES IN SCOTS PINE (*PINUS SYLVESTRIS* L.) IN THE CHERNOBYL EXCLUSION ZONE

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Plants are immobile organisms and thus must respond to changes in environmental conditions through physiological and evolutionary adaptation processes. The reactions of plants to abiotic stress are extremely complex processes, involving changes in mechanisms of gene regulation and expression, DNA methylation etc., leading to changes in viability or fertility and changes of genetic structures within populations. We studied genetic processes in Scots pine trees (*Pinus sylvestris* L.) that had been exposed to drastic environmental change caused by ionising radiation following the Chernobyl accident in 1986. Physiological adaptations and epigenetic effects were studied by observing gene regulation by means of RT-PCR of candidate genes and by an assessment of overall methylation levels in pines that had been exposed to different levels of acute and chronic radiation. Using microsatellite and AFLP markers we investigated mutation and selection in pine trees that have been growing in the vicinity of the nuclear power plant during the accident, in plantations that were established after the accident on soils exposed to radiation, and in reference material that had not been exposed to radiation. We present results on the respective roles of phenotypic plasticity, epigenetic effects and selection in the adaptation of pines to extreme environmental conditions caused by the Chernobyl accident.

Keywords: adaptation, epigenetic effects, *Pinus sylvestris*, selection

PREDICTING ADAPTIVE RESPONSES OF FOREST TREES FROM THE PERSPECTIVE OF EVOLUTIONARY ECOLOGY

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Present models of adaptive response of forest tree populations to environmental (climatic) change carry considerable uncertainties. While environmental change exerts directed selection pressure primarily on fitness traits, genetic information on this process is still scanty. The prominent role of phenotypic plasticity is also insufficiently explained. Genetic analysis of mature stands of sessile oak (*Quercus petraea* (Matt.) Liebl.) and provenance tests of beech (*Fagus sylvatica* L.) have been carried out to improve response modelling. Investigations in sessile oak populations in Hungary indicate that the selection pressure of climate extremes effectively shapes adaptive genetic variation at allozyme gene loci (e.g. *SKDH-A, GDH-A, ADH-A, AP-B*). In course of the “selective sweep” the frequency of certain alleles–presumably supporting drought tolerance–increased, which led to decrease of heterozygote rates and higher level of fixation towards the trailing (xeric) limits of distribution. Populations exposed to similar levels of selection pressure responded similarly, irrespective of geographic position. As rapid climatic changes in the next decades will affect first (presently existing) forest stands, their stability will greatly depend on the level of phenotypic plasticity. Analysis of beech in south-east European field tests illustrates not only the large potential of plasticity but support also the impression that the evolution of plasticity might be linked to climate conditions and intensity of selection. Populations of milder, maritime origin displayed higher plasticity than continental ones. The simultaneous effect of plasticity and directed selection presumably maintains an adaptive disequilibrium at genetic level, a clear analogue to the ecological disequilibrium at community level. This may partly explain the phenomenon of superiority of non-local populations over locally adapted ones. The existence of adaptive disequilibrium should be considered in models predicting response to environmental changes. If the se results are validated by other studies, these findings may have significant consequences also for the rules regulating the use of reproductive material in forestry, especially in climatically challenged regions.

Keywords: adaptive disquilibrium, climatic selection, extreme events, forest reproductive material, phenotypic plasticity

DISCUSSING THE ADAPTIVE VALUE OF DEVELOPMENTAL TRAITS IN MEDITERRANEAN PINES

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While the term adaptation is ubiquitous in current research on forest trees facing climate change, we still know too little about the contribution of single traits to total fitness in such long-lived organisms. Vegetative growth is commonly assumed to be a proxy of fitness when studying local adaptation of tree populations, but this may not be the case under moderate or severe environmental restrictions for plant growth. In particular, given well-known trade-offs between plant processes (e.g. growth, reproduction, defence), developmental phase changes are likely to be genetically based. Published data on various woody species indicate that both vegetative and reproductive phase changes are under strong genetic control and, at the same time, subject to wide ecotypic differentiation, which is meaningful from an evolutionary point of view. Mediterranean pines show widely differing life histories, both between and within species, but quantitative genetic data on key developmental aspects are so far very scarce. We conducted several field common garden experiments in Aleppo pine (*Pinus halepensis* Mill.), maritime pine (*P. pinaster* Aiton.) and Canary Island pine (*P. canariensis* C.Sm.), gathering data on plant size (height, diameter or stem volume) and survival in all species together with shoot ontogenetic status in *P. pinaster* and *P. canariensis* and female cone counts in *P. pinaster* and *P. halepensis*. Depending on the trial type (provenances, half-sib progenies or both), we used either population site interactions or genetic parameters (coefficient of additive genetic variance, strict sense heritability, Q_{ST} and genetic correlations) to separate genetic and environmental effects. Developmental traits such as vegetative heteroblastic change, threshold size for female reproduction and early reproductive allocation generally showed higher additive genetic variation and often higher genetic differentiation between populations than did vegetative growth itself. On the other hand, various genetic correlations between growth and reproduction (positive, null or negative) confirm contrasting life-history strategies with strong evolutionary implications.

Keywords: additive genetic variation, life-history, ontogeny, reproduction

CAN NUCLEOTIDE DIVERSITY PATTERNS IN MARITIME PINE (*PINUS PINASTER* AITON.) WOOD FORMATION CANDIDATE GENES BE EXPLAINED BY DEMOGRAPHY OR SELECTION?

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In perennial species, wood plays a key role in conducting sap and providing mechanical support and biotic and abiotic stress resistance. Its formation is a complex process involving several essential biochemical pathways such as lignin and cellulose biosyntheses. Although structural genes coding for enzymes of these pathways are well identified, far less is known about mechanisms that regulate the expression of those genes. We summarize nucleotide diversity results obtained on nine transcription factors (TFs) that are thought to play key regulatory roles in conifer lignification, using individuals from natural populations of maritime pine (*Pinus pinaster* Aiton.) from the French Atlantic coast. The diversity observed in the nine TFs was half that for other structural genes from the lignin pathway or other functional categories, which is consistent with the cis-regulatory evolution theory, which states that TFs should show little variation due to strong purifying selection. We also present coalescent simulations that model a large range of bottleneck scenarios to test whether patterns of diversity at each gene depart from neutral expectations based on the demographic history of the species. We then extend those results to a larger number of genes and to populations spanning *P. pinaster* distribution. We focus on contrasts across genes, and discuss the consistency of the best-fitting models with maritime pine species demographic history.

Keywords: nucleotide diversity, *Pinus pinaster*, simulation, transcription factors, wood formation

NUCLEAR MICROSATELLITES AND SNPs REVEAL LOW DIVERSITY IN STONE PINE (*PINUS PINEA* L.), A GENETICALLY DEPAUPERATED SPECIES

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Genetic variation is generally considered a prerequisite for adaptation to changing environments. Thus, the discovery of genetically depauperate but geographically widespread species is unexpected. In a first work, 12 paternally inherited chloroplast microsatellites were used to estimate population genetic variation across the full range of an emblematic circum-Mediterranean conifer, stone pine (*Pinus pinea* L.). We found that the same chloroplast DNA haplotype was fixed in nearly all of the 34 populations investigated. New data at the nuclear level (14 nuclear microsatellite loci and SNPs scored among about 280 genes) also show a near absence of genetic variation. We screened the literature to identify other cases of genetically depauperate plants. This search indicates that *P. pinea* is truly exceptional among widespread, sexually reproducing plant species for its low level of genetic diversity in both chloroplast and nuclear molecular markers. Stone pine appears to have passed through at least one severe and prolonged demographic bottleneck during the Quaternary, followed by subsequent natural- and human-mediated dispersal across the Mediterranean basin during the Holocene. Measurements from provenance tests indicate that the species harbours a non-negligible amount of variation for adaptive traits. This illustrates that although there is little doubt that genetic variation is the raw material for adaptation, the relationships between neutral diversity, quantitative trait variation, and adaptability are not straightforward.

Keywords: depauperate species, diversity depletion molecular markers, *Pinus pinea*

Parallel technical session H:

Migration under climate change

WHAT HAS DRIVEN THE SPREAD OF BEECH (*FAGUS SYLVATICA* L.) AND SPRUCE (*PICEA ABIES* (L.) H. KARST.) DURING THE HOLOCENE?

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Beech and spruce have achieved a dominant status over large regions of central Europe only during recent millennia, while several other tree species became abundant in different parts of Europe soon after the last Ice Age. We ask why their Holocene spread was apparently delayed and how their modern distribution relates to patterns of genetic diversity. We compare mapped networks of fossil pollen data with output from a climate-driven vegetation model and a dispersal simulator. We conclude that both species had early outlying populations but these did not act as significant foci for subsequent major population increases and do not contribute to the major patterns of current genetic diversity. Spruce responded more directly to suitable climatic conditions, particularly increased contrast between summer and winter temperatures, and spread across southern Scandinavia like a wave. Beech has also responded positively to the climates of recent millennia, but its north-eastern spread into Scandinavia was disjunct and frequently mediated by disturbance. There is increasing evidence for long-distance dispersal events and cryptic refugia but as yet less data about the genetic legacies of these phenomena.

Keywords: *Fagus sylvatica*, Holocene climate, migration, *Picea abies*, spreading rates

THE EFFECTS OF INBREEDING, GENETIC DISSIMILARITY AND PHENOTYPIC SELECTION ON MALE REPRODUCTIVE SUCCESS IN *SILENE LATIFOLIA* POIR., A DIOECIOUS PLANT

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Under natural pollination, pollen fate can determine genetic structure in species that occur in metapopulations with restricted gene flow and significant risk of inbreeding. Pollen fate can depend on mechanisms that mitigate the risk and costs of inbreeding and thus affect the persistence of small, isolated subpopulations. Inbreeding depression can enhance this effect, and favour outbred males, which often harbour recently introduced genes. To test for the effects on siring success of inbreeding and genetic similarity between breeding pairs, we established a population of inbred and outbred plants of *Silene latifolia* Poir., derived from controlled crosses. We exposed the plants to naturally occurring specialist pollinators and measured their phenotype. Siring success was determined by genotyping 29 females, 101 males and 752 offspring using five newly-developed nuclear microsatellite DNA markers. Outbred males sired significantly more offspring (1.61-fold increase in fertility) than inbred males. Significantly fewer offspring derived from full-sib matings than from matings among unrelated individuals, in agreement with results of hand pollinations. Independent of the effects of inbreeding, flower number and *in vitro* pollen germination were positively associated with siring success. These results indicate that a male's level of inbreeding and phenotype and non-heritable genetic effects of dissimilarity between mating partners affect pollen fate when plants are exposed to natural pollinators. These determinants of pollen fate will influence the structure of populations, generating few full-sib matings and reducing reproductive success of inbred males, thereby countering genetic erosion. These results are particularly important in the context of environmental changes that may lead to fragmentation of species distribution. Inbreeding avoidance mechanisms such as those we showed here might be crucial for the survival of species during these changes, by favouring external pollen flow, and therefore reducing the risk of local deme extinction.

Keywords: genetic similarity, inbreeding, pollen fate, siring success

THE IMPACT OF LANDSCAPE FRAGMENTATION ON POLLEN-MEDIATED GENE FLOW IN OAK SPECIES

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Landscape characteristics, including slope and elevation, (micro)climate, water availability, soil characteristics and anthropogenic land-use patterns, determine the distribution of populations in space and thus long-term gene flow characteristics. Extent and pattern of gene flow in turn are fundamental to the colonization capability of a species, and the persistence and evolutionary trajectory of its populations in time and space. In this study we compare fragmented mixed stands of pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.) in northern Germany, the centre of their distribution ranges, with small, isolated stands of *Q. robur* in the southern Ural Mountains, the eastern distribution limit of the species. The study included three oak stands 4 km from each other in Germany consisting of 200, 360 and 2200 mature trees and two relic stands (7 and 20 trees) on the eastern slopes of the Ural Mountains 80 km outside the species' continuous distribution range. The stands were fully inventoried and individual trees were genotyped at nine microsatellite loci. Stands were characterized using analyses of genetic diversity and population structure. Gene flow characteristics were established using paternity reconstructions and mating system analyses. In agreement with previous studies on oaks, the results indicate that mating and pollen-mediated gene flow occur mostly within stands. Still, a considerable fraction of pollination occurs between stands in both investigated regions. In the present study, successful pollen import can be differentiated into effective pollination between neighbouring stands and effective long-distance pollen transport. Effective long-distance pollen transport amounted to over 30% in the small, isolated stands on the eastern slopes of the Ural Mountains. Our results suggest that long-distance pollen transport can be effective, and small populations dispersed across the landscape seem to act as pollen traps. This finding is of particular importance to the evolution of populations that are fragmented, in suboptimal or nonstandard habitats or at range margins.

Keywords: landscape genetics, long-distance gene flow, pollen-mediated gene flow, population fragmentation

SENSITIVITY OF TREE MIGRATION RATE TO VARIATION IN DEMOGRAPHIC RATES AND THE PRESENCE OF COMPETITORS

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The pace of current anthropogenic climate change has raised concerns about the ability of plant species to respond through range shifts, as they did during the postglacial period. Recent genetic studies indicate that some species may be less dispersal-limited than has previously been thought. However, because competition and demographic stochasticity can reduce potential spread rates, and because global change may alter other demographic rates, it is not clear whether higher estimated dispersal distances truly translate into higher migration potential. In this study, we use a stand simulator model incorporating individual and year-to-year variation in growth, survival and reproduction and estimates of effective seed dispersal for northern red oak (*Quercus rubra* L.) derived from a hierarchical Bayesian parentage analysis incorporating genetic and ecological data to 1) analyse the sensitivity of migration rate to changes in several life history characteristics; 2) assess the significance of new genetic estimates of dispersal ability for potential migration rate; and 3) investigate the effect of competition on migration ability. We found that population growth and expansion rate are more sensitive to changes in dispersal than to proportional changes in fecundity or size at maturity, but demographic stochasticity and minor differences in initial conditions lead to wide variation in response. Competition with other forest species slows potential migration rate significantly relative to simulations without inter-specific competition. Even with higher estimates of dispersal ability based on genetic data and under best-case scenarios, maximum estimated migration rates for red oaks are slow relative to projected rates of climate change, suggesting that oaks (and species with similar dispersal mechanisms) may be at a disadvantage in reaching suitable future recruitment sites.

Keywords: climate change, competition, demographic stochasticity migration rate, simulation model, *Quercus rubra*

THE EFFECT OF POLLEN IMMIGRATION ON BUD-BURST PHENOLOGY OF OAK SEEDLINGS: DOES GENE FLOW AFFECT LOCAL ADAPTATION?

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Offspring resulting from long-distance pollen-mediated gene flow may exhibit different phenology to offspring of local matings. We investigated the bud-burst phenology of 2-year-old seedlings originating from a stand of about 5 ha composed of pedunculate and sessile oaks (*Quercus robur* L. and *Q. petraea* (Matt.) Liebl.) grown in a nursery. Local adults (421) and all seedlings were genotyped using five SSR loci, and 856 seedlings were assigned to one of 15 mothers. We found that 45.1% (±1.9%) of seedlings resulted from pollen immigration from outside the study stand, and seedlings from detectable pollen immigration were identified. ANOVA indicated that date of seedling bud burst varied significantly among the 15 half-sibs (F=13.8; P<0.001), but also that bud burst was later in seedlings sired by nonlocal paternal trees (F=8.02; P=0.005) than in those from local matings. Using a simplified additive model, we approximated the phenology scores of the mother trees, and then estimated the mean phenology scores of fathers responsible for pollen immigration within each half-sib. Pollen immigration was significantly (nonlinearly) related to bud-burst phenology of a given mother tree, with higher immigration rates observed among early and late mothers. However, the phenology of seedlings resulting from pollen immigration suggested that, while for early mothers immigrating pollen came from populations of distinctly earlier phenology, for late mothers the immigrating pollen originated from populations of similar phenology. We develop and discuss the theory that relates long-distance pollen-mediated gene flow and adaptation in forest trees.

Keywords: adaptation, gene flow, phenology, *Quercus*

GENETIC SIGNATURE OF A 150-YEAR-OLD POPULATION BOTTLENECK IN *FAGUS SYLVATICA* DESPITE RAPID POPULATION EXPANSION

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A major expected consequence of climate change is altitudinal and latitudinal shifts in the geographic ranges of plant species. These shifts and the associated demographic fluctuations have been shown to have pervasive consequences for genetic diversity. For instance, imprints of postglacial recolonization events on genetic structure can still be observed after millennia and at the continental scale. However, explicit empirical tests of how specific contraction/expansion processes may shape patterns of genetic diversity remain scarce. In particular, there are few studies addressing these processes at the scale at which dispersal and expansion actually take place and at which management guidelines are required, i.e. at the local scale and over several generations. This study takes advantage of a unique opportunity to investigate recolonization dynamics and their consequence for population genetic diversity in European beech (*Fagus sylvatica* L.) on Mont Ventoux, France, at an intermediate spatial and temporal scale employing a combination of historical records and genetic data. From the 12th century to the mid-18th century human activities reduced the beech forest on Mont Ventoux to a few remnant stands, whereupon forest management policy and practices changed to favour expansion of beech forest. Analyses of historical forest maps and management documents showed that beech refugia were not as scarce as has been reported. Since 1876 beech forests have expanded by as much as 65%, moving at an average rate of 35 to 42 m/year. Current patterns of genetic diversity and differentiation were investigated using 1825 individuals in 51 plots from three regions on Mont Ventoux genotyped at 13 microsatellite markers. The strongest differentiation was between plots (F_{plots}/total = 3.5%), with a surprisingly low level of differentiation between zones (F_{zone}/total = 0.9%). Isolation by distance was detected up to 750 m. The approximate Bayesian computation (ABC) method for estimating the probabilities of demographic scenarios based on microsatellite data supported the hypotheses of a bottleneck between 160 and 300 years ago, consistent with historical data. Hypotheses to explain the absence of differentiation between the northern and southern slope despite the bottleneck are discussed.

Keywords: bottleneck, colonisation, DIY-ABC, genetic structure, microsatellite

ESTIMATION OF CONTEMPORARY, 2030, 2060 AND 2090 CLIMATE FOR MEXICO, AND DESIGNING ASSISTED MIGRATION OF FOREST TREE SPECIES

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Planning assisted migration of forest tree species to realign genotypes to future climates requires prediction of climates that may occur. A spline climatic model was developed for Mexico, southern USA, Cuba, Belize, Guatemala and northern Honduras, using thin plate smoothing splines and the software ANUSPLINE. The model used normalized monthly means of temperature (average, maximum and minimum) and precipitation from nearly 4000 weather stations for the period 1961–1990 (‘contemporary’). The model was refitted for decades centred on 2030, 2060 and 2090 using monthly means updated from outputs of three general circulation models (Canadian Centre, Hadley Centre and Geophysical Fluid Laboratory) and several emission scenarios (A2, A1B, B1 and B2). Climatic variables of relevance for plant species distribution were estimated: mean average temperature, mean average precipitation (MAP), degree > 5ºC (DD%) and an annual aridity index (ratio of square root of DD% to MAP). Averaging results across models and emission scenarios, temperature was estimated to increase by 1.5ºC by year 2030, 2.3ºC by 2060 and 3.7ºC by 2090. Precipitation decreases on average by 6.7% by 2030, 9.0% by 2060 and 18.2% by 2090. Expected increase of aridity for Mexico suggests the need for a complex strategy, with assisted migration towards higher altitudes rather than to northern latitudes. Application of the model is illustrated by two examples. First, estimation of climate values for an assisted migration of forest tree species that distribute along altitudinal gradients (*Pinus oocarpa*, *P. devoniana, P. pseudostrobus* and *P. hartwegii* in Michoacán state), which suggested the need to move each population approximately 300 m higher in elevation in order to realign them to climate of year 2030. Second, estimation of present and future suitable habitats for *P. chiapensis*, an endangered species of fragmented and restricted distribution, suggest considerable reduction of suitable habitats by 2060.

Keywords: altitudinal genetic variation, assisted migration, climatic change, Mexican pines, spline climatic model

A FIRST LOOK AT ASPEN (*POPULUS TREMULA* L.) PHYLOGEOGRAPHY ACROSS EURASIA

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Extending from our studies of the two hybridizing European poplar species, *Populus tremula* L. and *P. alba* L., we are exploring phylogeographic patterns of *P. tremula* across Eurasia, which reflect migration in response to past (and possibly present) climate change events. The Asian distribution of *P. tremula* is much larger but probably not as dense and continuous as that in Europe. Phylogeography in Europe has been successfully reconstructed using chloroplast genetic variations. The *P. trichocarpa* (black cottonwood) chloroplast DNA, which has been sequenced to a depth of 400X, served as guidance for detecting polymorphic regions with the PCR-RFLP-electrophoresis technique. Tandem repeat structures in chloroplast genes form one source of variation that is easily analysed. These repeats change the hypothetical secondary structure of intron DNA, which may have some significance in interaction with nuclear factors. We now want to explore whether there was any interaction between aspen populations in Europe and Asia during the Pleistocene and Holocene, as the current geographic structure of *P. tremula* and *P. alba* chloroplast variants in central Europe gives evidence for gradual mixing of separate recolonization routes in the south, and a more blurred picture of continuous or clinal variation in the north. With the help of colleagues from Russia and Kyrgyzstan, we advanced our chloroplast studies into Central Asia. Preliminary results show that there is a gradual shift in haplotype frequencies when moving east, with some European types possibly crossing the Urals. In central Siberia, there are still some haplotypes present that can be found as far west as the Moscow region. Implications of these findings on migration events in Siberia and Central Asia, with their complex history of glaciations, are discussed.

Keywords: chloroplast genome, Eurasia, phylogeography, *Populus tremula*, Siberia

VARIABLE PATTERNS OF CONTEMPORARY GENE FLOW IN BEECH (*FAGUS SYLVATICA* L.) ACROSS EUROPE

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Common beech (*Fagus sylvatica* L.) is one of a few keystone tree species found across much of Europe. Forests dominated by beech are found over a remarkably wide range of environments and are often rich repositories of species diversity for plants, animals and microorganisms. The structuring role of beech in forest ecosystems is well recognized, even though the mechanisms underlying the ecosystems' stability and their adaptation potential are only starting to be understood. Using spatially explicit parentage models and microsatellite markers, we investigated patterns of contemporary gene flow as revealed by naturally established seedlings. The studies were conducted across a wide range of environments including natural old-growth forests in Poland, managed stands in Germany, populations in mountains in France and a low-density scattered stand in the Netherlands. Patterns of contemporary gene flow appeared to vary across populations. Pollen dispersal was similar for most of the stands (moderate pollen immigration rate, m_p=0.6, and fat-tailed dispersal kernels). However, pollen dispersal was lowest in the old-growth natural forest (low immigration rate, m_p=0.5, and an exponential dispersal kernel), while the scattered population in the Netherlands exhibited the most extensive pollen dispersal (high pollen immigration rate, m_p>0.7, and an extremely fat-tailed dispersal kernel). On the other hand, while seed dispersal was fairly restricted in natural and managed stands (low seed immigration rates and exponential dispersal kernels), it appeared to be high in one mountainous forest and the scattered population (seed immigration rate m_s>0.3 and fat-tailed dispersal kernel). Variable patterns of contemporary gene flow in beech indicate its ability to persist under climate change through migration and adaptation facilitated by gene exchange among populations. However, this potential may depend on the stand structure, as well as its age and location.

Keywords: adaptation, *Fagus sylvatica*, gene flow, parentage analysis, pollen dispersal, seed dispersal

Posters

Session A:

Population genomics

ISSR MARKERS REVEAL POPULATION GENETIC STRUCTURE OF HUNGARIAN LILAC (*SYRINGA JOSIKAEA*, OLEACEAE)

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Hungarian lilac (*Syringa josikaea* Jaq. f. ex Rchb.) is a shrub endemic to the Eastern Carpathians. It is a rare species of mountainous bogs and wetlands along creeks. The distribution of Hungarian lilac is highly fragmented, with several populations in Ukraine and western Romania. The aim of this study was to evaluate the genetic variability of the populations and to reveal genetic differentiation among them. We collected samples of 47 specimens from four populations in western Romania and two populations in Ukraine. Seven inter-simple sequence repeat (ISSR) markers were used. Based on a total of 77 unambiguous bands the results show that samples clustered according to the natural population structure, and each population has its distinct genetic character. Two of the Romanian populations are similar, which may result from their location by the same creek. The two Ukrainian populations sampled are also similar, which may also be explained by their close location. The five ISSR primers showed two small Romanian populations clustered close to the two Ukrainian populations, while the other two Romanian populations were distinct from all others.

Keywords: inter-simple sequence repeat, population genetic structure, *Syringa josikaea*

GENOMIC SEQUENCE AND SNP ANALYSIS OF THE NADP+ DEPENDENT IDH ENZYME GENE LOCUS IN A FOUR-SPECIES SYMPATRIC OAK COMMUNITY

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The NADP+ dependent IDH enzyme (E.C. 1.1.1.42) plays a key role in the citrate cycle of the metabolism of living organisms. As an isozyme, it has often been used in population genetic studies. In some cases it has been suggested that it might not be selectively neutral. Isozyme analysis of the IDH locus has revealed high differentiation between the oak species *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. In the present study, we identified and obtained the nearly complete 3.5 kb sequence of the gene encoding for NADP+-dependent IDH enzyme gene in a subsample of five samples per species from the four sympatric and closely related species, *Q. petraea, Q. robur*, *Q. frainetto* Ten. and *Q. pubescens* Willd. We investigated the total number of nonsynonymous substitutions (4) and an equal number of synonymous substitutions (single nucleotide polymorphisms; SNPs) detected, for the total sample set. The variation patterns revealed from the analysis of the sequence and the SNPs were analysed within and among species. Our results were also compared to the results obtained in a previous study by the application of neutral molecular markers on the same four-species sympatric oak community (cpPCR RFLP, nSSR).

Keywords genomic sequence, NADP+-dependent IDH enzyme, *Quercus* spp., SNPs

EXPRESSION OF GENES FOR COLD TOLERANCE AND BUD SET IN SCOTS PINE (*PINUS SYLVESTRIS* L.)

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Genetic variation for timing of bud set and cold tolerance were evaluated in a large association population of Scots pine (*Pinus sylvestris* L.) from Punkaharju, southern Finland, along with five other populations used as controls for clinal variation. The population displays wide genetic variation for bud set (mean 103 days, family effect 10.46 days, additive variance 41.84 days and phenotypic variance 84.64 days). The expression of some putative candidate genes previously described in other species as involved in the photoperiodic control of growth rhythm and cold tolerance were analysed in the association population and two other populations along the bud-set cline. For photoperiod treatments, seedlings of eight families from each population were grown in a greenhouse under natural photoperiod conditions and in growth chambers under constant light for three months before transfer to three different photoperiod conditions (20 h/4 h, 17 h/7 h and 8 h/16 h light/dark). For cold treatments, the same families were grown in growth chambers under constant light for two and a half months and divided into two parts. The first part was directly submitted to the cold treatments (+4°C for one week followed by -5°C for 48 hours) while for the second part, seedlings were allowed to set buds under short day conditions (8 h light/16 h dark) before treatments. Gene expression was analysed using quantitative PCR. The results of this expression study will be described in relation to the phenotypic variance already observed, and compared with earlier findings in *Picea abies*.

Keywords: bud set, cold tolerance, day length, Finland, *Pinus sylvestris, Picea abies*

DISTRIBUTION OF NUCLEOTIDE DIVERSITY IN SPANISH POPULATIONS OF THE NON-MODEL SPECIES, YEW (*TAXUS BACCATA* L.)

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Genomic approaches are increasingly used in non-model tree species to address demographic and adaptive questions, in order to get a better understanding of past evolutionary processes and current potential to face environmental changes. However, dealing with non-model species requires additional efforts, as it often implies developing specific genomic tools and working with natural populations. The interest in yew (*Taxus*) species lies mainly in conservation and biotechnology applications, including anticancer therapies based on specific *Taxus* metabolites (e.g. taxol). Taxane compounds are considered to be related to resistance to insect pests and pathogenic fungi, and thus their production may be affected by different environmental conditions. We have sequenced five genes coding for enzymes that catalyze the synthesis of important intermediate compounds within the metabolic pathway of taxol (TXS – taxadiene synthase; T5aH – taxadiene 5-alpha hydroxylase; TAT – taxadienol acetyl transferase; TBT – 2-alpha-hydroxytaxane 2-O-benzoyltransferase; DBAT – 10-deacetylbaccatin III-10-O-acetyl transferase) in five populations of *Taxus baccata* L. from contrasting environments in Spain (40 individuals). Marked differences in nucleotide diversity have been found among genes. More diverse genes showed a clear distribution of haplotypes and high levels of population differentiation, suggesting geographical structure of nucleotide diversity. Different neutrality tests indicated that the observed gene diversity has been determined by either demographic or selective processes depending on gene and population.

Keywords: genetic conservation, neutrality test, nucleotide polymorphism, taxol, *Taxus baccata*

USING MULTISPECIES DATA FROM SPRUCE (*PICEA*) TO MAKE INFERENCES ABOUT ADAPTATION

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Phenotypic traits that are subject to the selective whims of adaptation have been studied extensively in plants. But the patterns of diversity that adaptation causes in the underlying genotype is still a subject of much research. It is therefore important to understand how adaptation affects patterns of genetic variation. However, a beneficial mutation that fixes in a population will not be seen at the intraspecies level and may go unnoticed in many population genetic studies. Multispecies data adds information by revealing which mutations have fixed between species. This could simply be due to drift or it could represent adaptation to a novel environment experienced by one of the species. Resequencing multiple loci across a number of closely related species makes it possible to look at patterns of polymorphism and divergence in species subject to different environmental changes. In general trees, and more specifically spruce (*Picea*), show an abundance of polymorphisms that are shared across species; this could be due to their relatively recent divergence or to large ancestral effective population sizes. There are, however, loci that differ from this genome-wide pattern, lacking shared polymorphisms and having a number of fixed substitutions instead. These could represent loci where a beneficial mutation has occurred that has rapidly fixed in the population, eliminating any shared variation and resulting in a number of fixed differences. One such locus has been found which is homologous to *GIGANTEA* in *Arabidopsis* and so is thought to be involved in the circadian clock. This therefore has implications for the role of the circadian clock in adaptation to novel environments.

Keywords: adaptation, circadian clock, *Picea*, selection, spruce

DNA SEQUENCE VARIATION OF DROUGHT-RESPONSE CANDIDATE GENES IN CHILEAN CEDAR (*AUSTROCEDRUS CHILENSIS*)

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Chilean cedar (*Austrocedrus chilensis* (D.Don) Pic. Ser. et Bizzarri), a native conifer of the Andean–Patagonian forest, is characterized by a high adaptive potential for growing in marginal areas and good timber quality. The species grows over a wide area and under a wide range of rainfall (from less than 400 mm to more than 2500 mm average annual rainfall). This study assessed adaptive genetic variation at SNP level in candidate genes involved in response to drought stress. Partial and complete sequences of genes from maritime pine (*Pinus pinaster* Aiton) and loblolly pine (*P. taeda* L.), Japanese cedar (*Cryptomeria japonica* (L. f.) D. Don), mesquite (*Prosopis juliflora* (Sw.) DC) and *Arabidopsis* available in public databases were explored to design or select primer pairs. Amplified heterologous fragment sequences from *aqpA* and *aqpB*, *HAK3p*, *lp3-1* and *pal-1* were analysed. DNA samples were collected from 30 trees from each of three areas with different precipitation regimes: Llao Llao, Pedregoso and San Ramón. Twenty additional DNA samples from drought stress assays also were included in the analysis. About 716 sequences (sequenced in both directions) were obtained mainly from *pal-1, aqpA* and *aqpB* genes consisting of 600, 300 and 250 bp, respectively. We found a high rate of SNPs; an average of one SNP per 19 bp across coding and noncoding regions. Aquaporin genes showed the largest number of SNPs, while *pal-1* amplified fragments presented only a few. Average nucleotide diversity was moderate for pooled samples (π=0.00922) but varied 100-fold among single genes. Several neutrality tests were performed to identify candidate genes that might be under natural selection. Four genes gave negative values, but only those for *aqpA* and *lp3-1* were statistically significant. The nonsynonymous/synonymous mutation rate showed values of less than 1, suggesting divergence from neutrality. Possible causes are discussed. This is the first report on SNP variation in genes potentially involved in drought response in natural populations of *Austrocedrus chilensis*. The polymorphism discovered will be used for further evaluation of the adaptive role of genes through association mapping.

Keywords: adaptive variation, *Austrocedrus chilensis*, nucleotide diversity, SNPs

GENETIC DIVERSITY AT NUCLEAR LOCI IN SCOTS PINE (*PINUS SYLVESTRIS* L.) IN SCOTLAND

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In Scotland, Scots pine (*Pinus sylvestris* L.) is at the extreme north-western edge of its vast distribution. It is a keystone component of the remnant Caledonian forests. To search for the molecular signatures of local adaptation and population history, nucleotide diversity at 12 nuclear loci was studied in Scots pine populations across an environmental gradient in Scotland. At eight loci, diversity patterns were compared between Scottish and continental European populations adapted to different environments. Scottish populations showed a deficit of rare nucleotide variants (multilocus Tajima's D=0.316 vs D=-0.379) and differ significantly from mainland populations in allelic frequency and/or haplotype structure at several loci. Within Scotland, western populations showed slightly reduced nucleotide diversity (π_{tot}=0.0068) compared with those from the south and east (0.0079 and 0.0083, respectively) and recombination to diversity ratio about four times as high (ρ/θ=0.71 versus 0.15 and 0.18, respectively). At common loci, a similar level of nucleotide diversity was found in Scottish and European populations (θ_{sil}=~0.01) but less rapid decay of linkage disequilibrium was found in Scottish (ρ=0.0086±0.0009) than in mainland European populations (ρ=0.0245±0.0022). At two loci, significant differentiation was found of western Scottish populations from all others, whilst a third showed strong allelic dimorphism across all populations. It seems likely that Scottish populations experienced a severe-enough bottleneck during colonization to cause a skew in allelic frequency distribution. However, heterogeneity among regions suggests that subsequent admixture of different refugial lineages following colonization may also have played a role.

Keywords: adaptation, bottleneck, linkage disequilibrium, nucleotide diversity, population differentiation

EVALUATION OF GENETIC AND ADAPTIVE DIVERSITY IN CHESTNUT POPULATIONS USING SSRs AND EST-SSRs MARKERS

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Molecular markers such as microsatellites (SSRs) have been extensively used to study genetic diversity. However, while SSRs, due to their high variability and locus-specific nature, are useful in measuring neutral DNA variation, they are not useful for measuring adaptive variation. In contrast, EST-derived SSR markers, a new type of marker based on expressed genomic regions, can be used to measure functional diversity of species. Our research is focused on sweet chestnut (*Castanea sativa* Mill.), the only European species of the *Castanea* genus. This species is able to grow under a wide range of environmental conditions, and could thus offer a good model for study of adaptive diversity. The two principal aims of the current study were to: 1) study the information provided by both types of markers, and 2) assess neutral and adaptive variation in a set of European chestnut populations. Nine chestnut populations distributed throughout representative areas of contrasting climatic conditions in the Mediterranean basin were evaluated. Six SSRs and nine EST-SSRs from the EVOLTREE project data base were used. The Ewens-Watterson test was applied in each locus to assess selective neutrality. Both SSRs and EST-SSRs were polymorphic for all the populations analysed, although allelic variation was greater in neutral SSRs than EST-SSRs. AMOVA analysis showed that variation was greater within populations than among populations for both types of markers, but EST-SSRs indicated twice as much variation among populations as did SSRs. UPGMA and PCA analysis showed slightly different grouping of the populations for the two types of marker. These results indicate that the combined use of SSR and SSR-EST markers is a useful tool to understand genetic and adaptive diversity in chestnut.

Keywords: adaptive diversity, *Castanea sativa*, EST-SSR, genetic diversity, microsatellites, SSR

EVIDENCE OF GENETIC DRIFT IN NEUTRAL AND ADAPTIVE GENOME

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Genetic drift is a stochastic process that affects the entire genome, including genes involved in quantitative characters that are potentially adaptive. However, it is commonly seen as operating exclusively on neutral genes. To our knowledge genetic drift has never been measured in natural forest tree species populations, probably because all requisites for such measurement, i.e. at least two populations of common origin, selection pressure in the same direction and genetic isolation, are rarely fulfilled. We studied two pairs of neighbouring populations of the dioecious, wind-pollinated Patagonian cypress (*Austrocedrus chilensis* (D.Don) Pic. Ser. et Bizzarri) separated by 1200 m and 2600 m. Within each pair, populations lay on a north–south axis in a xeric environment and consisted of about 100 individuals, with approximately equal numbers of male and female trees. We reanalysed partial data from previous studies, including that on 26 allozymes from 12 loci, 207 alleles from 8 SSR loci, germination capacity, germination energy and early height growth curves in greenhouse trials, seedling survival and carbon isotope discrimination ($\delta^{13}C$) after one year in field trials, and apical damage and maximum photosystem II efficiency (F_v/F_m) after water stress in a greenhouse trial. Genetic drift and genetic isolation were shown by means of fixation and loss of alleles at three isozyme loci and 19 microsatellite private alleles with frequencies above 10% in one of the paired populations. Surprisingly, genetic and phenotypic values for most of the quantitative traits also differed between neighbouring populations, indicating that common origin and/or homogenizing selection have not counteracted the effect of drift. This study offers two important conclusions: 1) wind seems to act as a gene flow barrier in this case, and 2) adaptive genes can be also affected by genetic drift. These have consequences for the management of the genetic resources of any species in the current scenario of climate change.

Keywords: *Austrocedrus*, differentiation by drift, isolation by wind, small populations

LOOKING FOR SIGNS OF CLINAL ADAPTATION AND DEMOGRAPHIC HISTORY IN THE SEQUENCES OF SCOTS PINE (*PINUS SYLVESTRIS* L.)

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Clinal adaptation to environmental gradients is a well-known and long-studied phenomenon in many forest trees. In Scots pine (*Pinus sylvestris* L.), timing of bud set and cold hardiness in the autumn are examples of such adaptive characters. We sequenced 10 candidate genes for timing of bud set and cold hardiness in 10 populations of *P. sylvestris*; 7 populations to represent the variation in the main distribution of the species in Europe, and 3 additional populations that represent the variation at the range margins. We also studied the demographic history of *P. sylvestris* from a set of 32 reference genes using approximate Bayesian computation methods in a pooled sample of six European populations. We compared the fit of three different demographic models—standard neutral model, population expansion model and bottleneck model—and found that the bottleneck model best explains the data, which is in agreement with previous findings. Sequence variation in the candidate genes is examined against this background and with the viewpoint of clinal adaptation. On average, nucleotide diversities in candidate genes are similar across geographical areas; $\theta=0.00484$ in latitudes 66–67 °N, 0.00462 in latitudes 56–60°N and 0.00438 in latitudes 48–50°N. Our data show indications of allele frequency clines in some of the candidate genes. Frequency-spectrum reveals an excess of low frequency polymorphism in two of the candidate genes after correcting for demographic history. Further analyses of clinal selection are being conducted.

Keywords: candidate genes, clinal adaptation, demography inference, *Pinus sylvestris*, sequence variation

DEVELOPMENT OF FUNCTIONAL MARKERS FOR STRESS-RELATED GENES IN AUSTRIAN OAK (*QUERCUS ROBUR*, *QUERCUS PETRAEA*) POPULATIONS

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Long-lived trees such as oak face considerable environmental challenge from climate change. The genetic diversity of these populations has mostly been assessed using neutral markers such as microsatellites or AFLP. In the present study we developed an easy, high-throughput SNP detection system to assess genetic variability in genes related to drought/osmotic stress tolerance in oaks. Allelic diversity was established by three independent SNPs per gene, theoretically allowing the identification of eight alleles per gene locus if each SNP yields two variants. Genes encoding for betaine aldehyde dehydrogenase (BADH) and oxylase-like protein (OLP), which are highly upregulated in the root of sessile oak (*Quercus petraea (*Matt.) Liebl.) plantlets under osmotic stress, were analysed in 672 individuals from seven areas of Austria differing in climatic conditions. This revealed significant differences in allele frequencies. Genes that respond to drought stress were also identified in a 2-year glasshouse experiment and microarray analysis, including lipid transfer protein, late embryogenesis abundant 14, early responsive to desiccation 8, auxin responsive factor 16, open stomata 1, beta amylase 3/7, peroxidase 64, germin-like protein 3A and low expression of osmotically responsive genes 1. SNP markers for these genes are under development. Consistency between stress responsive genes identified in the microarray experiment and resequenced oak genes (INRA Pierroton) is currently being evaluated. This data will be used to develop an Illumina-Array for genotyping a large number of individuals at many loci.

Keywords: diversity, drought, *Quercus robur*, *Quercus petraea*, SNP

LINKAGE DISEQUILIBRIUM IN SWEET AND WILD CHERRY (*PRUNUS AVIUM* L.)

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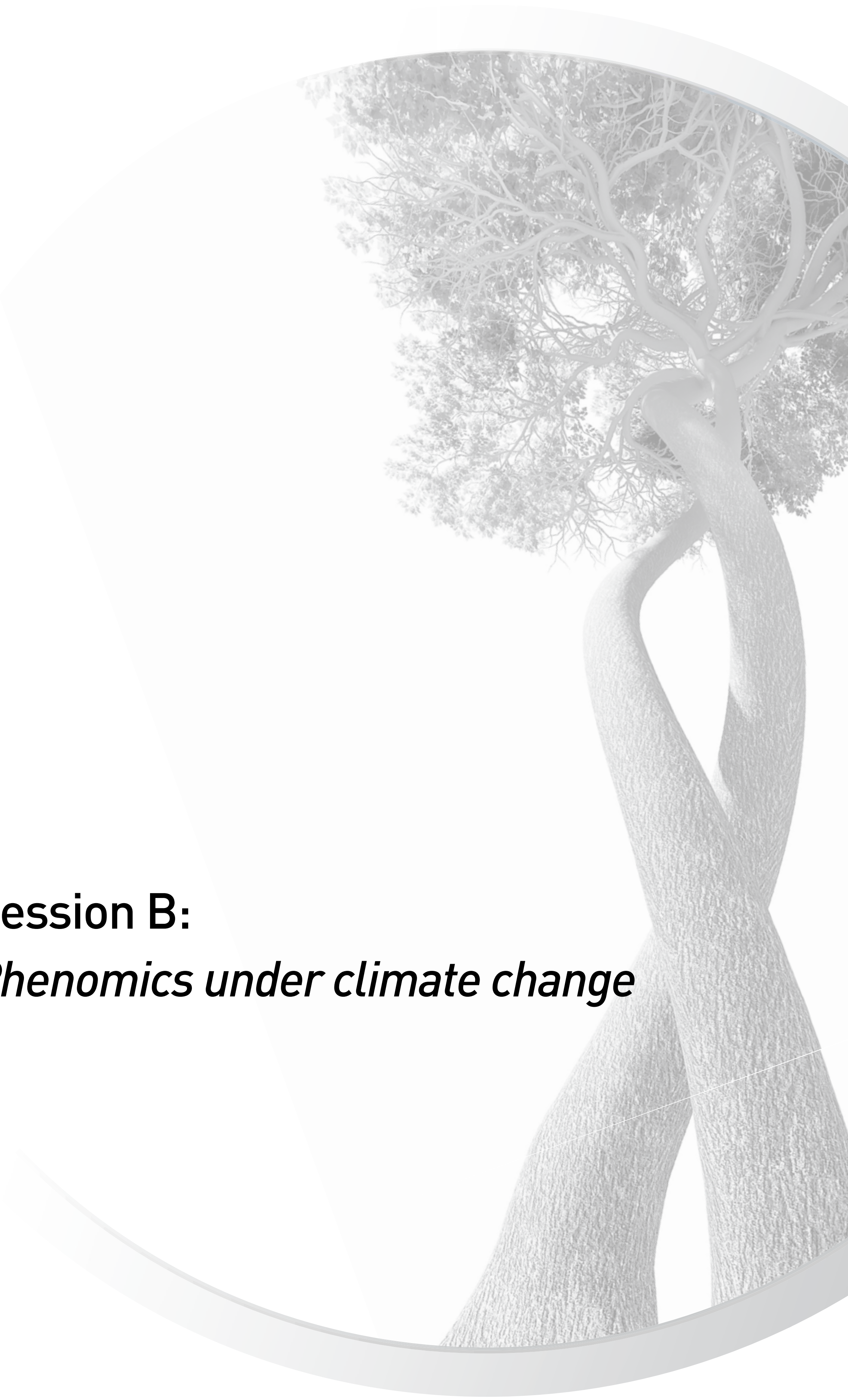
A prerequisite for association studies is the knowledge of linkage disequilibrium (LD) in the studied species. This study is the first to examine the pattern and magnitude of LD in the strictly self-incompatible cherry species, *Prunus avium* L.. We analysed LD in three gene pools (wild cherry, sweet cherry landraces and modern sweet cherry varieties) using a set of 35 microsatellite markers and the self-incompatibility locus. Since LD can be dramatically increased by genetic structure, we combined LD analysis with a detailed structural analysis on the different pools. Due to the bottleneck associated with domestication and breeding, we hypothesized a higher LD in the cultivated pool (sweet cherry) and a low LD in the wild pool (wild cherry). As predicted, significant LD was detected in sweet cherries (*D*'~0.250 in landraces and *D*'~0.295 in modern varieties) and a lower level was observed in wild cherry (*D*'~0.144). We also showed that the remaining structure in the group of landraces was responsible for a part of the detected LD. The results suggest that cultivated pools of *Prunus* may be studied for precise determination of the position of QTLs, whereas a rapid decrease of LD in the wild gene pools is promising for further association analysis at the SNP level. Our objective is to develop association studies in *Prunus avium*, particularly on candidate genes possibly implicated in genetic determinism of phenology.

Keywords: cultivated, linkage disequilibrium, *Prunus avium*, wild



Session B:

Phenomics under climate change

A DUAL PROTEIN PHOSPHATASE IS EXPRESED IN CHESTNUT STEM AMYLOPLASTS DURING WINTER DORMANCY

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Dormancy is an adaptive mechanism that enables woody plants to survive the freezing temperatures of winter. The process is complex and characterized by growth cessation, bud set, extensive metabolic remodelling, an acquired high tolerance to cold and, in deciduous trees, by leaf senescence and abscission. The protective role suggested for the oligosaccharides in cold acclimatization of herbaceous plants can be also important in cryoprotection of woody plants during winter dormancy. With the onset of autumn, the starch stored in stem and buds is broken down while at the same time there is a build-up of oligosaccharides, predominantly sucrose, trehalose, raffinose and stachyose. Initiation of starch degradation thus must be considered to be a key event involved in freezing tolerance associated with the dormant state. In recent years, a chloroplastic dual specificity protein phosphatase (DSP) was found to be involved in leaf starch metabolism in *Arabidopsis*. It has been stated that the *AtDSP4* gene is necessary for normal starch degradation and a role has been proposed for it in the protein phosphorylation/dephosphorylation mechanisms that modulate the activity of starch-degrading enzymes. Recently, it has been shown that *Arabidopsis DSP4* has a phosphoglucan phosphatase activity *in vivo* and has a role in starch catabolism. In our study we found that expression of *CsDSP* in the stems of adult chestnuts (*Castanea sativa* L.) increased at the beginning of autumn and the enzyme level remained high until onset of spring. The localization of the protein in the stem plastids during this period suggests the involvement of this enzyme in the starch degradation process that occurs during winter dormancy.

Keywords: *Castanea sativa*, cold acclimatization, dual protein phosphatase, starch metabolism, winter dormancy

GENETIC DETERMINISM OF WATER USE EFFICIENCY IN PEDUNCULATE OAK (*QUERCUS ROBUR* L.)

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Genetic diversity in intrinsic water use efficiency (W_i) has been studied within a pedunculate oak (*Quercus robur* L.) family. W_i is defined at the leaf level as the ratio between net CO_2 assimilation rate and stomatal conductance to water vapour. For large scale phenotyping, this trait can be estimated by measuring carbon isotope composition ($\delta^{13}C$) on organic material (leaves, wood). Genetic determinism for W_i was evaluated using a genetic mapping/quantitative trait loci (QTLs) approach and several regions were detected on the genetic map. Phenotypes of this family have been investigated intensively and partitioning of the observed diversity of W_i has shown a strong influence of stomatal conductance, whereby variation in W_i was related to variation in stomatal density and stomatal sensitivity to diurnal courses of irradiance. A modelling approach was used to detect more details of functional differences among genotypes. A complementary molecular biology approach was used to create a list of possible candidate genes related to the variability of W_i, using differential gene expression, as well as the study of the *ERECTA* gene, which is known to be related to variation in W_i in *Arabidopsis thaliana*.

Keywords: carbon isotope composition, stomatal conductance, stomatal density, water use efficiency

IDENTIFICATION AND EXPRESSION PATTERN OF MARITIME PINE (*PINUS PINASTER* AIT.) GENES UP-REGULATED BY PEG-INDUCED WATER STRESS

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Current climatic models forecast an increase of 1.5–4.0ºC in annual average temperatures over the next 100 years, together with disturbances in annual rainfall regimes. All these models agree that the Mediterranean basin may be dramatically affected. Annual rainfall is expected to decrease in this region and seasonal drought is expected to increase in depth and duration. Understanding of the molecular and physiological basis of drought stress response in Mediterranean forest species is thus of the utmost importance. Our model species, maritime pine (*Pinus pinaster* Ait.), is one of the most common conifer species in the western Mediterranean, widely used for afforestation in the Iberian Peninsula due to its ability to grow under poor conditions and across a wide range of environments. Notwithstanding its relatively small geographical range, the species shows large ecological amplitude, particularly in relation to rainfall regimes and water availability. We report here the identification of genes induced in maritime pine by polyethylene glycol (PEG)-induced water stress in hydroponic culture. We obtained and screened subtractive cDNA libraries (SSH). The putative function of selected genes was determined by homology with available sequences in public databases (GenBank, NCBI). Selected genes were included in a microarray to follow their stress-induced expression pattern. We have detected a total of 67 ESTs up-regulated during the treatment, which can be clustered in 5, 6 and 5 groups according to their expression pattern in roots, stems and needles, respectively. We have also applied a drought treatment on plants grown on soil, in order to follow expression patterns of genes under water stress similar to that faced in natural conditions.

Keywords: drought, microarray, *Pinus pinaster*, polyethylene glycol, SSH

VARIATION IN SEEDLING SURVIVAL IN NATURAL POPULATIONS OF PATAGONIAN CYPRESS (*AUSTROCEDRUS CHILENSIS* (D.DON) PIC. SER. ET BIZZARRI) UNDER DROUGHT STRESS

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The persistence of some forest tree species in the face of global climate change may depend on differences in drought tolerance among natural populations. The Patagonian cypress (*Austrocedrus chilensis* (D.Don) Pic.Ser. et Bizzarri) is found in the ecotone between the temperate southern beech (*Nothofagus*) forests and the steppe in southern South America. Any increase in drought conditions in the region would put the survival of its forest patches at risk. Inter- and intrapopulation variation of response to severe drought in this conifer was studied in a greenhouse pot experiment. One-hundred-and-twenty 3-year-old seedlings of each of eight natural populations (four from xeric habitats (<500 mm annual precipitation [a.p.]), two from humid conditions (>1300 mm a.p.) and two from mesic habitats) were grown under common garden conditions in three blocks. Ten open-pollinated families were represented in the two populations from the driest zone (330 mm a.p.). Watering was suspended for 43 days in late spring until half of the plants in the first block had died. Volumetric soil water content (VC, % vol/vol) was recorded once a week in five pots using TDR equipment. Pre-dawn water potential was measured in an extra sample of 20 plants each week. Maximum photosystem II efficiency (F_v/F_m) was measured weekly within this period in a sample of five plants per genetic entity (N=130). Survival was evaluated in the whole trial after a recovery period of 8 months during which all plants were 'normally' watered. ANOVAs were performed based on general linear mixed models for both F_v/F_m and survival data. Populations differed significantly for F_v/F_m (P=0.019) and survival (P<0.001), even those originating from the same precipitation zone. 'Family' significantly affected survival (P=0.001), while for F_v/F_m significant differences were due to only one family (P<0.001) which showed exceptional drought tolerance. The difference among populations from the same precipitation zone indicates the relevance of stochastic processes (such as genetic drift) in modelling genetic structure even of adaptive characters. Different survival mechanisms among families could be a conservative strategy to assure local evolution of populations.

Keywords: adaptation, *Austrocedrus*, chlorophyll fluorescence, drought stress

QUANTITATIVE TRAIT LOCI ANALYSIS OF RESOURCE-ECONOMY-RELATED TRAITS IN WILLOW (*SALIX* SPP.)

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Hardwoods, such as willow (*Salix* spp.), grown on agricultural land are a promising source of bioenergy. However, achieving high biomass yields with relatively low inputs of nitrogen fertilizer and irrigation water requires optimization of resource-use efficiency. The use of easily detectable genetic markers linked to genes underlying these traits would increase the efficiency of selection and breeding. This study examines the effects of water deficit and nitrogen supply on several resource-economy-related traits in an experimental cross of *Salix viminalis* L. and *Salix schwerinii* E. Wolf grown in pots under greenhouse conditions. Genetic variation and transgression were observed for all the traits. The genotypes that grew best in the water-deficit treatment were different from those that grew best under well-watered conditions. The genotypes with the best performance in the well-watered environment increased shoot growth largely by increased leaf nitrogen pool, whereas the genotypes performing best in the water-stress treatment enhanced growth largely by means of increased leaf nitrogen efficiency. We mapped quantitative trait loci (QTLs) for growth, biomass allocation, leaf area efficiency, leaf nitrogen content and leaf nitrogen efficiency by using a dense genetic linkage map previously developed for the same population and aligned to the poplar physical map. We found QTLs common to all treatments but also distinctive QTLs for different treatments. QTLs for the different traits were in some cases overlapping. The use of a genetic map aligned to the physical map of poplar allowed us to search the regions of the poplar genome corresponding to QTLs identified in willow for candidate genes putatively involved in the control of these traits.

Keywords: biomass, nitrogen use efficiency, QTL, water-stress, willow

THE STRAIGHTENING PROCESS AND STEM STRAIGHTNESS IN *PINUS PINASTER* AITON

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Stem straightness is a major focus of maritime pine (*Pinus pinaster* Aiton) breeding programmes. Despite the stability of performance in provenance trials, the efficiency of breeding programmes based on scoring stem forms remains low. An alternative approach for early selection is to analyse the biomechanical processes involved in the control of stem form, instead of evaluating form itself. The rationale is that genetic differences in biomechanical ability to straighten in young plants will be active in controlling stem form lifelong. Just as drought tolerance is not manifested in a moist environment, a gravitropic stimulus is needed to elicit the straightening process. In this study, the components contributing most to the genetic differences between provenances in the straightening process were analysed. The study employed a kinetic analysis and a biomechanical model that defines the non-linear interactions between the variables involved in the straightening process driven by secondary growth and reaction-wood formation (Fournier's model). This framework was tested on three *P. pinaster* provenances, selected for their differences in adult straightness and growth. One-year-old plants were tilted 45º and individual stem position and size were recorded weekly over 5 months. The radial extension of reaction wood and the anatomical features of wood cells were measured on serial cross-sections. From this, the integral effect of reaction wood on stem leaning was computed using Fournier's model. Responses driven by both primary and secondary growth were involved in the straightening process of the plants, but the latter were more significant in the differences between provenances. Plants from the provenance with greatest adult straightness showed greatest ability to straighten due mainly to 1) more efficient reaction wood (higher maturation strains) and 2) more pronounced secondary-growth-driven autotropic decurving than the less-straight-stemmed provenances. These two process-based traits are thus good candidates for early selection of stem straightness. This new approach is currently being tested on a greater number of genotypes.

Keywords: autotropism, gravitropism, maturation strains, provenances, reaction wood

COMPARATIVE GENETIC MAPPING BETWEEN OAK (*QUERCUS*) AND CHESTNUT (*CASTANEA*) SPECIES

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Quercus and *Castanea* are the Fagaceae genera most widely distributed across Europe. A set of 700 microsatellite markers was developed within the EVOLTREE project from a catalogue of 103 000 *Quercus* ESTs and was tested for amplification and polymorphism in both genera. A total of 319 and 133 markers were mapped on the reference *Quercus* and *Castanea* pedigrees, respectively. Comparative genetic mapping showed that macrosynteny was conserved, confirming that EST-SSRs are highly transferable markers between these two closely related species and very useful for comparative mapping. QTLs for adaptive traits (height growth, bud burst, water use efficiency) were also identified on the same pedigrees. Some of the QTLs for bud burst and height growth were found at homologous regions. We discuss these findings and how they are used to understand the genetic basis of forest tree adaptation.

Keywords: comparative mapping, Fagaceae, QTL, SSR

A FIELD EXPERIMENT TO STUDY THE PHENOTYPIC PLASTICITY OF MARITIME PINE (*PINUS PINASTER* AITON) SAPLINGS TO WATER STRESS

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Projections of climate change for Europe point towards more extreme precipitation regimes coupled with more intense, more frequent and longer lasting heat waves inducing frequent and severe droughts. Adaptation to water constraints is thus of increasing concern for wood production. In this study, phenotypic plasticity of maritime pine (*Pinus pinaster* Aiton) to water availability was quantified in the field over two years by planting one-year-old seedlings under a greenhouse open at its borders for rainwater exclusion. Water was provided to half the plants by aerial irrigation to compensate for evapotranspiration. The trial compared two half-sib families of the local improved provenance, one fast growing, the other slow growing. Soil water content, water table level, air temperature and humidity were monitored. Shallow soil water content decreased to 6% in the dry treatment in late summer. Predawn leaf water potential was regularly measured and reached -1.37 MPa in the dry treatment/slow-growth family at the end of experiment, compared with -0.2 MPa in the irrigated treatment. Plant height and diameter were measured three times per year (in March, July and September 2008 and 2009), following which between 40 and 70 saplings were uprooted for aerial and root biomass and architecture assessment. Various organs (bud, leaves, wood) were also sampled for anatomic, chemical and genomic analysis. Surprisingly, after one year of the trial, water shortage did not affect total biomass of the saplings. However, the water-stressed trees had slimmer stems, and had allocated more biomass to needles (+18%) and distinctly less to roots (30%), especially distal roots. After two years, the saplings of the fast-growing family were less tapered, and showed highest total height whatever the treatment. In the irrigated treatment, an increase of diameter growth and a greater resistance to cavitation were observed only at the end of the second year of experiment, showing a significant level of plasticity for the fast-growth family for these two traits. We hypothesize that *Pinus pinaster* saplings stop root growth when the soil is too dry, but maintain their productivity by setting more needles.

Keywords: phenomics, *Pinus pinaster*, plasticity, water-stress

Session C:

New technologies in ecosystem genomics

IDENTIFICATION OF OAK SPECIES FROM DRY WOOD AND ITS APPLICATION FOR WINE MATURATION

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The majority of aromatic compounds in wine are directly induced during maturation by the contact with oak wood from barrels, staves or wood chips. For example, whisky lactone (3-methyl-4-octanolide), which gives a woody taste, is easily detected and appreciated by consumers. Sessile oak (*Quercus petraea* (Matt.) Liebl.) and pedunculate oak (*Quercus robur* L.), the two major European oak species used for wine maturation, have very different aromatic contents, especially of whisky lactone. Identifying the species used in making barrels will facilitate the wine maturation process, for instance by providing wineries with more homogenous batches of barrels. The objective of our study is to identify oak species directly from dry wood, using molecular markers that will be applicable in an industrial context. Unfortunately, dry wood is dead tissue in which DNA is highly degraded and difficult to access. To optimize DNA recovery from dry wood, we first developed a quantitative PCR protocol based on chloroplast DNA to evaluate the efficiency of DNA isolation protocols. We then identified and developed molecular markers (EST-SSRs and SNPs) adapted to dry wood that are particularly diagnostic. Using an optimized DNA isolation protocol and these powerful markers, we were able to identify the species from wood samples that had been dry for two years.

Keywords: degraded DNA, *Quercus* spp., wine maturation

EXPLOITING NEXT-GENERATION SEQUENCING TO OBTAIN A WHOLE-GENOME SNP COLLECTION IN BLACK POPLAR (*POPULUS NIGRA* L.)

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Poplar trees can be found in both domesticated and wild environments. This situation provides extensive experimental opportunity for the study of the relationship between naturally occurring genotypic and phenotypic variation. Having access to a population with little or no human disturbance means that extant populations are the result of natural evolutionary forces and questions pertaining to adaptation and demography will not be anthropomorphically confounded. The genus *Populus* is useful as a model tree species, since many genetic and genomic resources are available, including mapping pedigrees, association populations, genetic and QTL maps and the genome reference sequence of the black cottonwood, *P. trichocarpa* (Torr. & Gray). Taking advantage of these resources and exploiting rapidly-evolving new sequencing technologies, we set out to produce a sequence-marker map made of SNPs and short indels covering the whole genome. Such data will be used as markers for association studies and genomic-based breeding for adaptive traits (e.g. phenology, leaf development, water use efficiency and rust resistance). This work focused on black poplar (*P. nigra* L.), the native and most wide-spread poplar species in Europe. A combined process was adopted to resequencing various *P. nigra* genomes. The first step was deep-coverage resequencing (≥ 20X) of three poplar individuals from different latitudinal origins, which provided for efficient SNP detection. Alignment of 75 and 100 bp sequences to the *P. trichocarpa* genome reference seems to work well even though two different species are being compared. This part of the work provided consensus sequences of *P. nigra* and a wide-genome SNP collection. This SNP detection was supported and confirmed by *P. nigra* sequences of candidate genes for the above-mentioned adaptive traits, which were resequenced using the traditional Sanger method. The consensus sequences obtained will be used in the second step of the process to align sequences from 50 additional individuals in order to maximize SNP discovery. These poplar genotypes have already been selected from various European natural populations and their lower coverage (2X) resequencing is in progress. A final discussion on sequence data analysis and marker distribution across the poplar genome is provided.

Keywords: next-generation sequencing technologies, *Populus* genome, SNP discovery

TRANSCRIPTOME ANALYSIS OF BUD DORMANCY IN SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIBEL.) BY 454 PYROSEQUENCING

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The timing of bud flush affects the fitness of individual trees. Status of bud dormancy changes from autumn to spring. In order to clarify the molecular basis of dormancy, we analysed differential gene expression between two dormancy phases (endodormancy and ecodormancy) in sessile oak (*Quercus petraea* (Matt.) Liebl.) buds using pyrosequencing. We sampled mRNA in October and February from two populations that differ in bud flush phenology. We constructed four cDNA libraries (two dormancy phases × two populations) and performed pyrosequencing using a Roche 454 FLX sequencer, which produced a total of 495 915 reads. Read sequences were cleaned up and duplicate reads were removed. Remaining sequences were mapped onto the OakContigV1 unique sequence collection which consists of 222 671 sequences (available from http://genotoul-contigbrowser.toulouse.inra.fr:9092/*Quercus*_robur/index.html with user name <oak> and password <*Quercus*33>). This was constructed by assembling transcripts from 34 cDNA libraries for *Q. petraea* and pedunculate oak (*Q. robur* L.). The number of reads that were mapped onto each OakContigV1 sequence were counted and analysed using R and BioConductor statistical packages. We found 54 unigene elements in OakContigV1 that showed significant differences in the level of expression (read abundance) between the endo- and ecodormancy phases at the false discovery rate of 5%. Among these, 21 sequences (39%) had similarity with protein sequences in SWISSPROT databases. Sequences with similarity to cold shock protein and dehydrin were common. Four GO terms (response to stress, response to endogenous stimulus, secondary metabolic process and response to abiotic stimulus) were identified as significantly enriched. Genes identified in this analysis are suitable candidates for validation by qPCR.

Keywords: bud dormancy, gene expression, gene ontology, *Quercus petraea*

CANDIDATE GENE MAPPING IN PEDUNCULATE OAK (*QUERCUS ROBUR* L.)

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Candidate genes (CGs) involved in three adaptive traits for oak (bud burst, drought resistance and hypoxia related traits) were mapped in an intraspecific cross of pedunculate oak (*Quercus robur* L.). We compared different genotyping methods based either on SSCP (single strand conformation polymorphism) using silver staining, capillary (ABI) and Li-Cor DNA sequencer or primer extension (Veracode Illumina, fluorescence polarization detection [FP-TDI]). Out of the 192 CGs tested, 94 were mapped on the 12 linkage groups of the genome. Gene distribution across linkage groups was rather heterogeneous, ranging from 1 gene on LG4 to 24 genes on LG2, in agreement with the result also found for 350 mapped EST-SSRs. QTLs were projected on the newly constructed male and female maps. This made it possible to detect coincidences between CGs and trait QTLs. We will illustrate such co-location for a QTL for epinasty (downward bending of leaves) and an ethylene responsive element binding protein (EREBP).

Keywords: candidate genes, genotyping methods, oaks

A WEB PORTAL FOR OAK GENETIC AND GENOMIC DATA

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Over the years various genetic and genomic databases were developed on oaks, hosted on various servers. There was clearly a need to make all the information held in these databases rapidly and efficiently available through a user-friendly informatics tool, despite its heterogeneity. We created a web application, called Quercus Portal, that aims to facilitate the exploitation of the large amount of available data. Quercus Portal provides download links, general information and direct access links to the databases of interest. Moreover, it provides a dynamic web interface allowing users to undertake queries across all databases simultaneously. Entries to the query system are targeted towards various concepts we defined in order to help the user to easily and accurately select elements from the hundreds of thousands available, including genetic units, genetic markers, phenotypic traits and tissues. Elements of the concepts can be combined to search for biologically relevant responses. This web interface is plugged into the EVOLTREE eLab cache database. The databases included in the Quercus Portal are: QuercusMap + Cmap, 10 genetic maps and QTL data of mapping pedigrees; (GD)^2, georeferenced database of genetic diversity which contains data originating from genetic surveys conducted over the natural distribution of the species; Treepop, phenotypic and genetic data of association populations; Oak provenances, passport data of provenances and provenance tests across Europe; EPA, a clustering and annotation pipeline for oak ESTs; SSRs, genomic and genic microsatellites; and GnpSNP, a database of SNPs. While some of the data are limited to EVOLTREE partners, much is publicly available.

Keywords: database integration, genetics, genomics, oak, web application

ANALYSIS OF EXPRESSED SEQUENCE TAGS IN MARITIME PINE (*PINUS PINASTER* AITON)

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Maritime pine (*Pinus pinaster* Aiton) is an important species for the forestry industry in south-western Europe. A breeding programme for this species started half a century ago and has now reach its third generation. Early indirect selection criteria, particularly molecular markers and genes, are now being investigated to develop varieties with improved wood quality and adapted to more restricted water availability. With the main objective of discovering economically and ecologically important genes, we developed an EST resource from (i) four cDNA libraries (needles, roots, differentiating xylems and buds) and (ii) constructed five subtractive suppressive libraries using total RNA extracted from differentiating xylem associated with different types of wood. With the exception of the SSH libraries, a mean of 10 000 sequencing runs were performed per library. Unexploitable chromatograms and quality checking led us to eliminate 9 096 sequences (22%). Finally, 31 678 ESTs were obtained and used in the assembly step (stackPACK™ software, available at http://www.sanbi.ac.za). Of these, 22 431 sequences were members of one of the 4483 primary consensus sequences found, with 9247 remaining as singletons, corresponding to a redundancy of 56%. A functional annotation (gene function and metabolic classification) was then assigned to each consensus sequence and singleton. This genomic resource provides a tool to study the maritime pine transcriptome and investigate functional diversity in natural populations.

Keywords: EST, genomic resource, maritime pine, *Pinus pinaster*

DEVELOPMENT OF A BAC LIBRARY AND BAC END SEQUENCING FOR PEDUNCULATE OAK (*QUERCUS ROBUR* L.): NEW TOOLS FOR OAK GENOME STUDIES

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Pedunculate oak (*Quercus robur* L.) is a model forest tree species for the study of the molecular, genetic and physiological mechanisms involved in adaptation. To facilitate molecular characterization of adaptive traits, a BAC library was constructed from one reference *Q. robur* genotype (3P). High molecular weight DNA was partially digested with EcoRI. The average clone insert size was 135 kbp, varying from 111 kbp to 170 kbp. Based on the haploid genome size of 780 Mbp/C, the BAC library was estimated to contain approximately 12 genome equivalents, which gives a greater than 99% chance of discovering at least one positive clone with a single-locus marker. Genome coverage, quality and utility of the library were further confirmed by PCR screening on 127 pool plates (representing 7 genome equivalents) using a set of 60 EST-SSR markers that span the *Quercus robur* 3P linkage map. On average, 7 pools were identified per marker screened. A first set of 15 000 BAC clones was sequenced at both extremities, leading to 23 737 high-quality BAC-end sequences (BES) with an average read length of 623 Q20 nucleotides. BLASTn searches against organelle DNA of poplar and grape indicated that there was 2% chloroplast contamination and 1% mitochondrial contamination. Approximately 5.1% of the BES contained potential transposable elements, most of which were retrotransposons (4.81%). A total of 2789 microsatellites was discovered within the BES. Dinucleotides TC and AT were the most abundant, accounting for 37.7% of all simple sequence repeats. These first results show that this BAC library provides a useful resource for screening genomic regions of interest and support future work of whole genome sequencing.

Keywords: BAC library, BAC end sequences, *Quercus robur*

GENERATING CANDIDATE GENE RESOURCES IN NON-MODEL CONIFERS: THE CRIEC INITIATIVE

The CRIEC consortium, coordinated by CIFOR-INIA (Santiago Gonzalez-Martinez[1]) and IGV-CNR (Giovanni G. Vendramin[2])

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In the framework of the EVOLTREE EU Network of Excellence, the CRIEC consortium has promoted the transference of conserved candidate genes that are expressed under different environmental stresses to a wide range of European conifers, including European yew (*Taxus baccata* L.), Mediterranean cypress (*Cupressus sempervirens* L.), common juniper (*Juniperus communis* L.), incense juniper (*J. thurifera* L.), Spanish fir (*Abies pinsapo* Boiss.), various species of cedar (*Cedrus*), Aleppo pine (*Pinus halepensis* Mill.), Turkish red pine (*P. brutia* Ten.), Italian stone pine (*P. pinea* L.), black pine (*P. nigra* J.F. Arnold), Canary Islands pine (*P. canariensis* C. Sim.) and Bosnian pine (*P. heldreichii* Christ). Candidate genes were first identified on loblolly pine (*P. taeda* L.) ESTs at David Neale's lab in the University of California, Davis (ADEPT2 and CRSP projects). Then, a set of about 300 candidate genes that were previously shown to transfer from New World pines (*Pinus taeda*) to Old World pines (maritime pine [*P. pinaster* Aiton] and Scots pine [*P. sylvestris* L.]) were collectively selected based on transferability, expressional profile, annotation and levels of polymorphism and sequenced in the CRIEC species panel. In this poster, we present first results on transferability rates and levels of nucleotide diversity based on supposedly orthologous candidate genes. We also provide first insights on phylogenetic relationships for Mediterranean pines based on a large gene set and on patterns of selection across species. Finally, examples of the applicability of these new resources for population genetics and evolutionary studies are provided.

Keywords: candidate genes, conifers, genomic tools, SNPs, transference

Session D:
Eco-regional trends in adaptation

GROWING LARGE LEAVES FROM A SMALL-LEAF GENE POOL: EVOLUTIONARY TRAJECTORIES AND MORPHOLOGY IN BLACK POPLAR (*POPULUS NIGRA* L.)

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The major river systems of Europe have been heavily modified by anthropogenic factors, causing significant changes to the riparian plant communities. Black poplar (*Populus nigra* L.), a forest tree endemic to the river floodplains of Europe, western Asia and northern Africa, is declining due to habitat loss and hybridization with domestic poplar varieties. Changes in temperature and precipitation predicted for the next century are expected to further impact *P. nigra* populations through changes to flooding patterns. *Populus nigra* trees display remarkable variation in phenotype across western Europe, with trees in Spain displaying small leaves and a branching architecture, and trees from the Netherlands south to northern Italy having large leaves and higher biomass. These differences are consistent with the phenotypic divergence of putative glacial refugia located in the arid Iberian Peninsula and the moister Italian Peninsula and Balkan regions of Europe. Further, trees from France are morphologically intermediate to the small-leafed and large-leafed morphotypes, but are genetically more similar to the Spanish samples, indicating that these populations likely arose from the Iberian refugia but have been under selection for the large-leaf phenotype of central Europe. If morphological traits reflect adaptation to precipitation patterns, changes in summer rainfall predicted for central Europe may favour the small-leaf morphotype, changing the evolutionary trajectory of this important forest tree. Combining morphological, physiological and genomic data from *P. nigra* collected across Europe has revealed the patterns of evolution of these complex, adaptive traits in natural populations. Future work involving transcriptomic profiling and whole genome scans will provide insight into the genomic and evolutionary consequences of the introgression of the *P. nigra* refugia in central Europe.

Keywords: adaptation, differentiation, morphology, multivariate analyses

DETERMINANTS OF MALE AND FEMALE REPRODUCTIVE SUCCESS IN EUROPEAN BEECH (*FAGUS SYLVATICA* L.): IT IS SIZE THAT MATTERS

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In hermaphroditic plants, including the majority of temperate forest trees, individual reproductive success can be achieved through male or female functions. There are numerous theories attempting to explain why particular individuals are more successful than others. Theories on sex allocation and trade-offs between growth and reproduction are well established in plant population biology, but they are weakly documented in forest trees. To investigate these phenomena we studied reproductive patterns among 300 *Fagus sylvatica* L. seedlings naturally established within study plot of ca. 3 ha consisting of 277 adult trees. Analyses used nine microsatellite loci and the seedling neighbourhood model, thus allowing for simultaneous assessment of male and female reproductive components. The analysis was carried out assuming typing errors (by means of estimable error rates), thus accounting for possible mismatches between genotypes when computing Mendelian probabilities. Individual male and female reproductive success was related both to ecological features such as distance and direction of propagule (seed or pollen) dispersal and to individual fecundity-related traits such as tree biomass and tree height. Seed immigration was low (m_s=1.5%) and not significantly different from zero. Up to 59.8% of seedlings had fathers located outside of the mother's neighbourhood, indicating extensive pollen immigration from sources more than 100 m away. Average distance of seed dispersal was low (8.0 m; 95% confidence interval, 7.3–8.6), while within the local population pollen travelled on average 51.2 m (33.2–267.3). Seed dispersal followed a thin-tailed exponential-power dispersal kernel and was weakly anisotropic. No signs of anisotropy were found for pollen dispersal, which followed a fat-tailed exponential-power kernel (though not significantly different from a simple exponential). The above-ground biomass of a tree appeared to be the most significant trait determining male and female reproductive success. Biomass of the most successful females was about 0.75 standard deviations larger than the population mean. Tree height was weakly positively correlated with male reproductive success, but significantly negatively correlated with female reproductive success, suggesting trade-offs between male and female reproductive functions. We found no evidence of selection acting jointly on biomass and tree height for either male or female reproductive success.

Keywords: *Fagus sylvatica*, gene dispersal, seedling neighbourhood model, selection gradients, sexual selection

GENETIC STRUCTURE AND VARIABILITY OF PHENOLOGICAL FORMS OF PEDUNCULATE OAK (*QUERCUS ROBUR* L.) FROM CLONAL SEED ORCHARDS IN CROATIA

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Mass selection of pedunculate oak (*Quercus robur* L.) in Croatia started in the 1980s and 150 plus trees were selected within three breeding zones. Three clonal seed orchards (CSOs) were established with grafted plus-trees. A clonal trial was planted with ramets from the majority of those trees and significant differences in flushing phenology were observed between the clones, with 20 days difference between the earliest and latest flusher. Bud breaking is an adaptive trait under strong genetic control. Variation in flushing phenology is presumably one of the species' defensive strategies against late frosts. There are some reports of significant differences in genetic variability and differentiation among phenoforms within temperate tree species, e.g. late-flushing forms of European beech had a higher intrapopulation genetic variability than early-flushing forms. The aim of this study was to compare variability within and among CSOs and investigate the genetic structure of clone phenoforms from the CSOs. Flushing phenology was assesed on a 1–7 scale (1 - dormant buds; 7 - fully developed leaves). Flushing phenoforms were determined based on clones' flushing stage on 18 April 2008. Genetic variability and structure were analysed at eight polymorphic SSR loci. Total number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosity and PIC for each locus, average number of alleles (N_{avg}), H_o and H_e, allelic richness (N_{ar}) and number of private alleles (N_{pr}) in each CSO and each phenoform were calculated. N_{ar}, H_o and H_e among CSOs and among phenoforms were compared using the Kruskal-Wallis test. Genetic differentiation among CSOs was estimated by F_{ST}. The distance matrix for the proportion of shared alleles was subjected to AMOVA to partition total SSR diversity into among and within CSOs and phenoforms. Both CSOs and phenoforms were not significantly different for N_a, H_o and H_e, and there were no significant differences in genetic differentiation among phenoforms. It seems that neutral markers analysed were not associated with adaptive differences between phenoforms. Additionally, the results indicate the absence of genetic differentiation between the breeding zones which raises an argument for the reconsideration of their existing delineation in Croatia.

Keywords: genetic differentiation, microsatellites, oak, plus trees, selection

THE ASSESSMENT OF GENETIC DIVERSITY AND PROGRESS ON DEVELOPING OF MICROSATELLITE MARKERS AND cDNA IN FOREST TREES AND WILD PLANTS IN THAILAND

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The current status of forest genetic resources must be understood before efficient conservation programmes can be designed. The status of genetic resources of forest tree and wild plant species can be explored by investigating the extent of genetic variation and the mating system in each species. The status of genetic diversity of some economically and ecologically important forest tree and wild plant species in Thailand has been studied using molecular markers such as RAPD, AFLP and SSR as well as isoenzymes. The results showed that genetic diversity and mating system varied among species. For example, Merkus pine (*Pinus merkusii* Jungh. & de Vriese) had low genetic diversity (H_e=0.058) and relatively low outcrossing rate (t_m=0.017–0.843) while teak (*Tectona grandis* L.f.) had high genetic diversity (H_e=0.310) and high outcrossing rate (t_m=0.872–0.995). Among the dipterocarps, hairy-leaf apitong (*Dipterocarpus alatus* Roxb. ex G. Don) had moderate genetic diversity (H_e=0.0924). In mangrove forest, *Rhizophora apiculata* Blume also had unexpectedly high genetic diversity (H_e=0.316) and a highly variable mating system among populations and families (t_m=0.241–0.978); *R. mucronata* Lam. also had high genetic diversity (H_e=0.385). High genetic diversity was found in wild plants, namely wild bamboo (*Bambusa bambos* (L.) Voss) and wild orchid (*Paphiopedium exul* (Ridl.) Rolfe), with values of 0.369 and 0.301 respectively. However, the genetic differentiation among populations (F_{ST} and theta P) of those species ranged from 0.0821 to 0.250. Strategies for conservation of the genetic resources of these forest trees and wild plants in Thailand will require various combinations of *in situ* and *ex situ* approaches. To determine the genetic diversity of other medicinal and endangered forest tree species, microsatellite (SSR) markers were developed in neem (*Azadirachta* spp.), emblic (*Phyllanthus emblica* L.) and Thailand rosewood (*Dalbergia cochinchinensis* Laness.). cDNA of genes related to enzymes involved in lignin biosynthesis in teak and genes related to ascorbic acid synthesis in emblic is under development. The possibility of the discovery of other functional genes in forest trees in Thailand is discussed.

Keywords: cDNA, forest trees, genetic diversity, microsatellite markers, wild plants

DOES SPATIAL GENETIC STRUCTURE INCREASE WITH ALTITUDE? AN ANSWER FROM NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.) IN TYROL, AUSTRIA

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Harsh environment at high altitude may affect the mating system of plant species, especially those with wide ecological amplitude. These changes will be reflected in different spatial genetic structure (SGS). For the first time, populations of Norway spruce (*Picea abies* (L.) H. Karst.) were analysed along an altitudinal cline to test the hypothesis of an increase in SGS with altitude. Three putatively autochthonous populations in Tyrol, Austria, at 800, 1200 and 1600 m above sea level (asl) were studied. Six highly polymorphic DNA markers (EST-SSRs) were used to genotype a total of 450 contiguous trees (150 trees per subpopulation). Nearly identical allelic frequencies were found, resulting in very small subpopulation differentiation ($F_{ST} = 0.002$) and suggesting a single population along the cline. The fixation index decreased with dbh (age), indicating natural selection against inbred trees. Loiselle's kinship coefficient was used to quantify SGS. Against expectation, no significant SGS was found in any of the plots, indicating a random spatial pattern. Significant SGS was observed when all plots were treated as a single population conforming to an isolation-by-distance pattern.

Keywords: Norway spruce, nuclear microsatellites

HAVE YEW (*TAXUS BACCATA* L.) POPULATIONS BEEN ABLE TO ADAPT TO THEIR ENVIRONMENTS?

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Most tree species show high levels of gene flow, large effective population sizes and are able to quickly adapt to local conditions. English yew (*Taxus baccata* L.) is a dioecious long-lived gymnosperm presently found throughout most of the European continent, often in small isolated populations. Accordingly, and despite the longevity and migration potential of this species, neutral genetic diversity in yew is strongly structured in the western Mediterranean, reflecting the strong impact of bottlenecks and genetic drift, particularly in its south-eastern (Mediterranean) range. While reduced gene flow is expected to favour genetic differentiation among populations, either neutral or adaptive, demographic bottlenecks and genetic drift are expected to reduce genetic diversity. In this scenario, have yew populations been able to get adapted to the environments where they are currently found? As a first step to answer this question we are studying the performance of 109 clones from 29 localities covering 6 contrasted climate regions of the Iberian Peninsula growing in a common environment. Preliminary results based on shoot growth show significant differences among localities and regions. An ecoregional trend exists linked to winter temperature, provenances from colder localities performing better than those from warmer localities. Differences in growth may be related to phenology and/or resource use efficiency. Future research will be focused on studying the variability among provenances in phenological behaviour, as well as anatomical and physiological traits.

Keywords: adaptation, neutral genetic structure, phenotypic variability, *Taxus baccata*

Session E:

Mitigation options

IS THE HIGHER GENETIC VARIABILITY IN NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.) SEED STANDS COMPARED TO THAT OF NON-SEED STANDS CORRELATED WITH SEED-STAND SELECTION CRITERIA?

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Selected seed stands in Slovenia are approved on the basis of their origin, distance from a stand of unknown origin or of lower quality, population size, developmental phase, homogeneity of traits at the time of production, health condition, three characteristics aimed primarily at assessing wood production, and adaptation to ecological conditions measured by nine criteria ranging from survival of natural regeneration to capability of regenerating after stress. The seed stands are evaluated in comparison with all other forest stands in the region. For Norway spruce (*Picea abies* (L.) H. Karst.) the minimum size of a seed stand is 5 ha, with at least 70 pollinating and seed-bearing trees, distributed at 1–2 tree-height spacing. Autochthonous origin is not obligatory in the case of Norway spruce. In order to compare genetic variability between seed stands and non-seed stands of Norway spruce, seven nuclear microsatellite loci were examined in seven seed stands and five non-seed stands in Slovenia; 27 to 35 mature trees or seedlings, each grown from seed originating from a single tree, were analysed. On average, the number of alleles was 7% higher in seed stands than in non-seed stands and the number of effective alleles was 3% higher. The number of private alleles in seed stands was on average twice as high as in non-seed stands. Based on these results seed stands have greater genetic variability. However, analysed seed stands occupied a 700 m altitudinal range and were of autochthonous and non-autochthonous origin while non-seed stands were all autochthonous and occupied a 400 m altitudinal range. Therefore a more systematic sampling strategy is needed to confirm these preliminary results.

Keywords: genetic variability, Norway spruce, seed stand, selection criteria, Slovenia

MAINTAINING GENETIC DIVERSITY OF FOREST GENETIC RESOURCES FOR ADAPTATION TO CLIMATE CHANGE

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Climate change is a major factor threatening human society, and forests are considered an important means of counteracting climate change. This study investigates the impacts of climate change on distributions of major forest tree species using a GIS-based niche modelling approach and explores approaches to responding to climate change through wider use of forest genetic diversity. The impacts of climate change on distributions of two typical tree species native to China, Chinese red pine (*Pinus massoniana* Lamb.) and China fir (*Cunninghamia lanceolata* (Lamb.) Hooker), were investigated using two sets of climate data, current and projected for 50 years in the future. Results indicated that the predicted ranges most suitable for the two species will decrease and be more fragmented, possibly due to alteration by warming temperature of the unique ecological niches required by the species. Plasticity and resilience of trees were used to explain the adaptation of forest to climate change and its relation to genetic diversity. High genetic diversity provides more opportunities for trees to adapt to climate change. A new approach to selecting trees for adaptation was illustrated using genetic diversity of populations rather than individuals. Patterns of deployment of trees can also improve the capacity of adaptation to climate change.

Keywords: adaptation, climate change, forest genetic resources, mitigation

INTRAPOPULATION VARIABILITY OF THE PROGENY OF SILVER FIR (*ABIES ALBA* MILL.) PROVENANCES FROM THE POLISH CARPATHIANS

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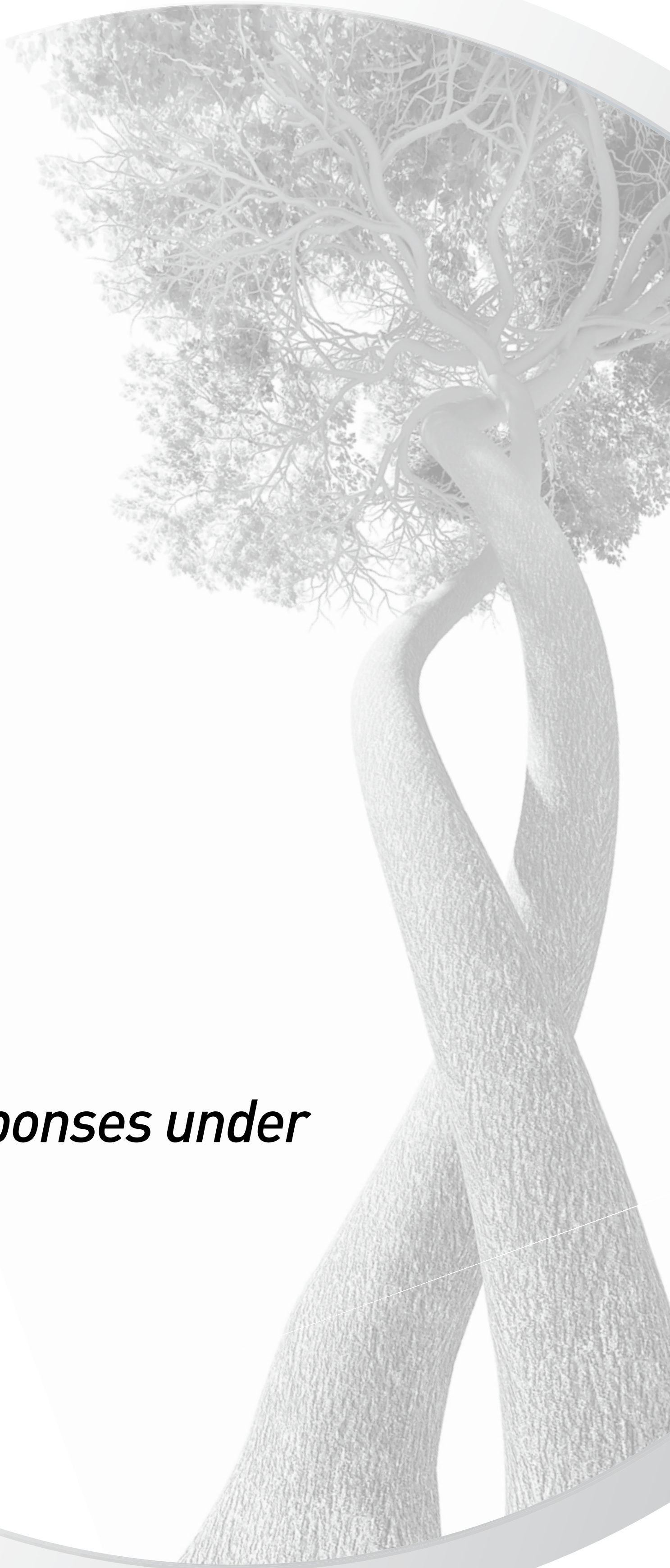
The study investigated intrapopulation variation of 41 provenances of silver fir (*Abies alba* Mill.). Traits measured included those related to adaptability, morphology and resistance traits, as well as total height, root-neck diameter and spring growth of fir at juvenile age. Results showed that differences in adaptability, phenological and morphological traits were genetically determined. They also confirmed the significance of provenance × environment interaction. The study identified the best-performing populations under local conditions, which could be used in early tests.

Keywords: *Abies alba* Mill., adaptive traits, progeny testing



Session F:
Community responses under climate change

INCORPORATING HERBIVORE–PLANT INTERACTIONS INTO A POPULATION DYNAMICS MODEL OF THE GREEN OAK LEAFROLLER (*TORTRIX VIRIDANA* L.)

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The population dynamics of many species is not only affected by intraspecific relationships (e.g. competition and mating) but also by interspecific interactions (e.g. predator–prey and herbivore–plant). Whereas strong interspecific interactions (e.g. predator–prey) are well understood and many models have been developed for them, weaker interspecific interactions (e.g. herbivore–plant) have not yet been subject of intensive investigations or modelling. Here we propose a model for the population dynamics of the green oak leafroller (*Tortrix viridana* L.) regulated by varying food quality depending on larval densities. *Tortrix viridana* is a forest pest species on oaks in central Europe. Mass outbreaks, which can cause complete defoliation, occur in a periodic sequence every six to eight years. We developed a density-dependent matrix model with four stages that included various survival rates and food requirements. It is well known that cycles with a period of six or more generations can only arise via negative feedback with a two-generation delay. We hypothesize that this delayed negative feedback could be caused by herbivore-induced change in leaf quality of the host tree. There are two possibilities of how this quality change could work. On the one hand a decrease in leaf quality could be a direct reaction to strong herbivory (inducible defence response), with the influence of low leaf quality being transmitted to the next generation through maternal effect. On the other hand there could be a delayed reaction to strong herbivory in terms of a low leaf quality in the subsequent year. In feeding experiments we will investigate which form of regulation (or both) is realised in *T. viridana* populations. The results of this experiment will be the basis for a parameterization of the matrix model in order to create realistic population cycles. In future, we plan to simulate population dynamics of *T. viridana* at the landscape level. Low genetic differentiation between different populations suggests high dispersal activity. The extension and parameterization of the model with genetic data will enable a simulation of dispersal activity of *T. viridana*.

Keywords: modelling, population dynamics, *Tortrix viridana*

THE BIOGEOGRAPHIC HISTORY OF THE HOST TREE MATTERS FOR ITS ECTOMYCORRHIZA COMMUNITY AND SEEDLING PERFORMANCE

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Organisms within communities not only coexist, but interact and co-evolve. Evolutionary processes in foundation species such as trees influence the structure of associated communities. Species interactions influence genetic and phenotypic processes of the participating species. This interdependent correlation of co-adaptive progress is more intensive when the interaction of partners is close, such as in ectomycorrhizal symbiosis (ECM). Therefore, provenances of a given tree species that have been separated for a long period should show distinct differentiation in population genetic structure, which might be partially due to co-adaptive evolutionary processes and different abilities of successful mycorrhization with the ECM community in a chosen environment. A well-adapted ECM interaction would have an effect on growth and fitness of the host. We performed a translocation experiment in the Black Forest (Germany) where we exposed three seed provenances (local: Black Forest; foreign: Macedonia and Romania) of silver fir (*Abies alba* Mill.) to the local ectomycorrhizal community. After one growing season the young firs exhibited a large number of ectomycorrhizal taxa. The local provenance revealed both the highest mycorrhization rate and the highest fungal richness; it also had the highest root and shoot biomass. Since the chosen tree provenances belong to two ancient isolated genetic lineages we analysed population genetic structure for differentiation. SSR analysis provided a clear separation of the lineages, i.e. the Black Forest provenance was different from the Balkan provenances. Searching evidence for co-adaptive progress of local silver firs and the ECM community, we performed an ANCOVA which showed higher plant performance through higher mycorrhization. Indication of genetic imprints correlated with associated ECM pattern comes from a principal component analysis: we compared host-tree genetic variability (single locus genotypes) with fungal variability. The local silver firs clearly span a broader range for both variables than the foreign provenances. Although no direct causal relationship is at hand we suggest that the local provenance may profit from a broader spectrum of ectomycorrhizal fungi.

Keywords: *Abies alba*, community genetics, ECM, population genetics, seed provenances

SELECTIVE PRESSURES BETWEEN OAKS AND THEIR OBLIGATE FUNGAL PARASITE CAUSING POWDERY MILDEW

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Progress in understanding plant-pathogen evolutionary dynamics requires integration of molecular approaches with studies of diseases in populations. We initiated several studies directed towards this goal, taking oaks, a model tree species for population and evolutionary genetics, and their most common disease, powdery mildew caused by *Erysiphe* spp., as a study system. A wide range of traits potentially involved in the interaction, and their genetic determinants, were considered, including both probability of host–pathogen contact (phenological synchronization) and compatibility (resistance and defence in trees, virulence and aggressiveness in fungi). A first experiment focused on tree–fungus phenological synchrony along an altitudinal gradient. We showed that synchrony between tree bud burst and fungal spore discharge in spring varied along the gradient and within populations. Within-stand variation in tree phenology was associated with infection escape. The pathogen was not locally adapted for phenology at population level. A second experiment was set up to test the Janzen-Connell hypothesis at an infraspecific level, more specifically the potential role of powdery mildew in the maintenance of spatial genetic diversity in oak stands. A prerequisite was to test the level of host specificity in fungal strains. Preliminary results in a controlled experiment suggested higher pathogenicity of fungal strains on seedling progenies from their tree of origin than on other progenies. A third experiment was set up to test the real-time effects of selection by the pathogen on its host. A reconstructed oak population consisting of 15 pooled progenies was sown as acorns in an experimental design with three disease pressure treatments: natural, increased (inoculated) and decreased (fungicide treatments). Material was taken from each plant for DNA extraction. Individual oak seedlings will be monitored for survival and performance over three years from 2009. In parallel, a set of several hundreds of functional resistance/defence candidate genes was defined. The potential effect of disease selective pressure on host-tree genome will be assessed by genotyping 1500 SNPs in these genes and comparing genetic structure in the initial population and in the surviving subpopulation (or between high/low performance individuals).

Keywords: co-evolution, disease resistance, host–pathogen, phenology, selection

STRUCTURE OF ABOVE- AND BELOW-GROUND POPULATIONS OF THE ECTOMYCORRHIZAL FUNGUS *LACCARIA AMETHYSTINA* (HUDS.) COOKE IN A BEECH FOREST

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Ectomycorrhizal fungi may play a key role in the adaptation of tree populations to changing environments since they promote water and nutrient uptake in plants. We need to improve our knowledge of the structure of ectomycorrhizal communities and populations and relationships between host and fungi to better understand the potential adaptation of forest ecosystems to new environmental conditions. In this study we focused on beech (*Fagus sylvatica* L.), a keystone tree species in Europe, and *Laccaria amethystina* (Huds.) Cooke, one of the most frequent symbionts of beech. Fruiting bodies and mycorrhizae of *L. amethystina* were mapped and collected in autumn 2008 and 2009 in several plots located in a beech forest within the EVOLTREE Intensive Study Site at Blizyn, Poland. All fruiting bodies and mycorrhizae collected were genotyped using nine different SSR markers and the genetic structure of the populations was analysed. Results show a very high genetic variability within *L. amethystina* populations at the fruiting-bodies level, with populations formed by numerous small genets (or genetic individuals). Almost every single fruiting body corresponded to a unique genet, with a maximum genet length of 1.5 m. Results also show a quick renewal of individuals, with almost no genets persisting from one fruiting season to the next or even within a single season. The propagation of the fungus by sexual reproduction implies that each year the host is exposed to new genets that may have different abilities. Analyses of the genetic structure of mycorrhizae are on going. Results should allow us to determine if the same degree of genetic variation is observed on the roots and to compare the population structure above and below ground. Preliminary results suggest that genets forming the mycorrhizal root tips are not the same as the ones that are fruiting. Genotyping of the beech trees these mycorrhizae are associated with should also allow us to determine the specificity of *L. amethystina* genotypes toward the host genotype and the role of this fungus in the establishment of beech seedlings.

Keywords: beech, mycorrhiza, population genetics, SSR markers

GENETIC DIVERSITY AT CANDIDATE LOCI POTENTIALLY INVOLVED IN PHENOLOGY IN NATURAL POPULATIONS OF TWO LEPIDOPTERA SPECIES

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Phenology relates to the timing of the main steps of the biological cycle, such as reproduction, egg hatching, larval instars and pupation. Variability in phenology can have a very high adaptive value, as it may be directly associated with different environmental conditions (temperature, photoperiodism) or to synchrony with host development. Our goal was to study the potential genetic basis of phenology of insect species related to oaks (European oak leafroller, *Tortrix viridana* L.) or pines (pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermüller) using a candidate gene approach. This was made possible by the development of transcriptomic resources through EVOLTREE. Ecological studies have shown that phenological plasticity is one of the main traits explaining the geographic distribution of the pine processionary moth, with a delayed reproduction (late summer and fall) in regions with hot and arid summers and an early reproduction (early summer) in northern latitudes and higher altitudes. One population from Portugal is known for its atypical biological cycle. This natural mutant is thus a useful model to investigate the genetic basis of this major phenological shift. The European oak leafroller is an oligophagous moth strictly associated with the genus *Quercus*. Its development is influenced by the phenology of host trees; the first instar larvae feed on newly opened buds and later immature stages depend on the developmental expansion and growth rate of the leaves for survival. Previous ecological studies revealed that *T. viridana* populations exhibit different phenologies depending on their host species (e.g. pedunculate oak [*Quercus robur* L.], Turkey oak [*Q. cerris* L.] or downy oak [*Q. pubescens* Willd.]). For instance, a population feeding on *Q. pubescens* was found to hatch 7 to 10 days earlier than the sympatric population feeding on *Q. cerris*. We identified from the scientific literature ca. 20 candidate genes involved in development and phenology of insects. Transcriptomic sequences for each studied species were screened to identify these genes. Whenever possible, specific primers were designed to amplify and sequence them in natural populations exhibiting contrasting phenologies. Preliminary results are presented, which describe the structure of some of these candidate genes, as well as the genetic diversity observed in natural populations.

Keywords: candidate genes, phenology, re-sequencing, *Thaumetopoea pityocampa*, *Tortrix viridana*

INFLUENCE OF GENETIC VARIATION IN SCOTS PINE (*PINUS SYLVESTRIS* L.) AND SITE WATER REGIME ON NEUTRAL AND FUNCTIONAL DIVERSITIES OF ITS MYCORRHIZAL ASSOCIATE, *CENOCOCCUM*

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Episodes of drought in Europe have increased in frequency and duration during the 20th century and climate change scenarios predict that this trend will continue. Climate change has already altered global patterns of forest biodiversity, which may induce fundamental shifts in species interactions. We studied how soil water regime acts as the determining abiotic factor shaping patterns of biodiversity, in particular the interactions between Scots pine (*Pinus sylvestris* L.) and the associated ectomycorrhizal (ECM) fungus *Cenococcum geophilum* Fr. Enhanced drought resistance is considered one of the important functions of ECM symbiosis for host trees and *C. geophilum* is known to be especially important in this respect. We addressed two main issues: 1) Is there a structuring role of genetic variation within Scots pine on the ECM community in general and the genetic and functional diversities of *C. geophilum* in particular? 2) What is the impact of site water regime on the ECM community and the genetic and functional diversities of *C. geophilum* associated with Scots pine? We addressed these questions by studying natural populations in four intensive study sites along a latitudinal cline representing a drought gradient across Europe, and by two field experiments, one with various planted families of Scots pine and one with irrigation in a dry Scots pine forest. We used nuclear microsatellites to assess the genetic diversity of host trees and associated *C. geophilum*, and (pyro)sequencing of the ITS region from ECM roots to determine the ECM community. To study functional aspects of the symbiosis and the impact of drought, we used transcriptomic and proteomic approaches to measure gene and protein expression in both host tree and mycorrhizal partner root tips. Our findings, such as the observed change in the ECM community after irrigation, add to our knowledge of how forest trees and their associated organisms adapt to variation in the environment and to each other. This will help us predict how forest ecosystems might respond under future global changes.

Keywords: *Cenococcum geophilum*, community genetics, ectomycorrhizal diversity, gene expression, *Pinus sylvestris*

IMPACT OF SOIL WATER REGIME ON GENE EXPRESSION IN THE MYCORRHIZAL FUNGUS *CENOCOCCUM GEOPHILUM* FR. ASSOCIATED WITH SCOTS PINE (*PINUS SYLVESTRIS* L.) AND IN FREE-LIVING MYCELIA

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Current climate change scenarios predict that episodes of drought will increase in frequency and persistence in Europe, which may induce fundamental shifts in patterns of biodiversity and species interactions. In dry, inner-alpine regions such as Valais in Switzerland, Scots pine (*Pinus sylvestris* L.) forests are already changing, with pine showing increased mortality rates and deciduous species such as downy oak (*Quercus pubescens* Willd.) becoming more abundant. Increasing drought is thought to be the main reason for this shift. Enhanced drought resistance is one of the fundamental benefits of ectomycorrhizal symbiosis. The ascomycete *Cenococcum geophilum* Fr. is known to be particularly effective in this respect, mainly by protecting the absorbing roots from desiccation. This species seems to be more resistant to drought than others and is commonly becoming more abundant on roots exposed to drought. We studied how *Cenococcum* responds to changing water regimes by analysing its gene expression in free-living mycelia under sterile conditions and in *Cenococcum*/Scots pine mycorrhizae. The mycorrhizae were sampled in a field experiment with irrigation located in a dry Scots pine forest in Valais, Switzerland. To study the transcriptome changes in free- living mycelia, *Cenococcum* was grown in Petri dishes on agar medium and exposed to drier conditions by either opening the Petri dishes or by increasing the agar concentration in the medium four-fold compared with the control. In total, we constructed five cDNA libraries; two using RNA extracted from mycorrhizae (control/irrigated) and three from RNA of free-living mycelia (control/open dishes/4× agar). The cDNA libraries were tagged and pyrosequenced, producing 410 708 expressed sequence tags (ESTs). From these, 12 600 consensus sequences (contigs) were identified and compared with the non-redundant GenBank database for homology searches. Comparing the presence and abundance of ESTs in the different cDNA libraries will allow us to identify candidate genes implicated in drought resistance and species interactions. Studying these genes may help explain how organisms associated with forest trees adapt to variation in the environment and how species interact under changed conditions.

Keywords: *Cenococcum geophilum*, drought, ectomycorrhiza, gene expression, species interactions

A PROTEOMIC APPROACH TO ASSESSING THE ROLE OF ECTOMYCORRHIZAE IN DROUGHT RESISTANCE

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Ectomycorrhizae (ECM) are believed to play a critical role in resistance and resilience mechanisms of forests. Although the overall beneficial effect of the symbiosis between ectomycorrhizal fungi and their tree hosts has been well studied, the fine process details associated with role of ECM in various mechanisms such as drought resistance is poorly understood. Proteomic approaches have great potential for providing new insights on ECM-related processes at the molecular level, but there is very little information on the application of such methods. The objective of this research was to study the effects of drought stress on functions of ectomycorrhizal roots at the level of their expressed proteins. For this purpose, two different proteomic techniques were established: the soluble fraction and extents of posttranslational modifications were studied using 2-D electrophoresis, and the hydrophobic protein fraction was examined using tandem mass tags. To study effects of different site water regimes on proteome fractions of *Cenococcum geophilum* Fr. and *Rhizopogon roseolus* (Corda) Th. Fr. associated with Scot pine (*Pinus sylvestris* L.), mycorrhizal samples were taken at the EVOLTREE intensively studied site at Valais, Switzerland. The optimized methods and their value in assessing the soluble and cell-wall associated protein fractions are discussed.

Keywords: *Cenococcum geophilum*, drought resistance, *Pinus sylvestris*, proteomic approach, *Rhizopogon roseolus*

Session G:
Evolutionary responses under climate change

GENETIC STRUCTURE VS HEALTH STATE OF NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.) STANDS IN POLAND

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The development of molecular techniques has led to significant improvement in our knowledge of plant genetics and understanding of the molecular mechanisms operating within plant genomes. Molecular markers have been used extensively in the identification of the genotypes of many forest tree species and in monitoring genetic variation across Poland. In the present study we investigated the genetic structure of Norway spruce (*Picea abies* (L.) H. Karst.) populations. Eleven populations from the north of Poland and eleven populations from the south were examined and characterized using four nuclear microsatellite (nSSR) markers. Parameters of genetic diversity (Nei's H) and differentiation (G_{ST}) were estimated and correlated with defoliation degree in the stands. Microsatellite data revealed an overlapping of genotypes as a result of past human impacts on Norway spruce stands, such as the effects of harmful gas emissions, e.g. NO_2 and SO_2. Healthy trees were more genetically diverse than those that were losing their leaves, demonstrating their greater adaptability to harmful environmental conditions.

Keywords: defoliation index, genetic differentiation, nuclear microsatellite DNA markers, *Picea abies*

ANCIENT HYBRIDIZATIONS BETWEEN EURASIAN ASHES ARE CORRELATED WITH MAJOR CLIMATIC AND GEOLOGICAL VARIATIONS

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In forest trees, large contact zones usually exist as a result of broad geographical ranges, high outcrossing rates and long-distance pollen flow. Because the speciation history and phylogeography of the section *Fraxinus* in Europe remain unclear, we have been studying natural hybridization and reticulate evolution between common ash (*Fraxinus excelsior* L.), narrow-leaved ash (*Fraxinus angustifolia* Vahl), which are indigenous to France along fluvial basins such as the Loire and Sâone valleys, and Manchurian ash (*Fraxinus mandshurica* Rupr.), an east Asian species. To study the extent of genetic differentiation among *Fraxinus* species, a wide sample of diverse populations of European ash taxa was collected throughout their ranges, together with the related Asian ash. AFLP markers and nETS and nITS sequences of rRNA nuclear genes were used to reconstruct the history of these species using NeighbourNet and STRUCTURE analyses, as well as Bayesian and maximum likelihood approaches. The phylogeny for the group was partially congruent with that previously published, despite natural hybridization being observed in the European species. However, a notable incongruence was observed between nETS and nITS phylogenies, which was confirmed by AFLP analysis. These patterns were interpreted as a consequence of ancient hybridization between *F. angustifolia* and *F. mandshurica*. A dated reticulate phylogeny was then reconstructed, showing that speciation and reticulation events could be traced back to the Miocene period (24–5 million years ago), and were correlated with major geological events inducing climate changes in Eurasia. These results indicate that interspecific hybridization has been a recurrent factor in the history of the group, induced by climatic and geological changes as driving factors for allopatric speciation in Eurasia.

Keywords: *Fraxinus*, hybridization, phylogeny, reticulate evolution

JOINT EVOLUTION OF TWO QUANTITATIVE TRAITS IN CONSTANT AND CHANGING ENVIRONMENTS

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Variation in correlations among functionally related traits can influence survival and reproduction under environmental change. A better understanding of the joint evolution of adaptive traits depends on a knowledge of their functional architecture, in which pleiotropy has long been recognized as an important component. We modelled a metapopulation composed of 25 populations connected by gene flow in an island situation. Individuals in these populations were characterized for two quantitative traits. Each trait was coded by 10 quantitative trait loci (QTLs), some of which may be pleiotropic. We compare the evolution of genetic differentiation for the quantitative traits (Q_{ST}) and the QTLs (F_{ST}) as an indicator of the intensity and mode of selection. We assumed first a constant environment, where each population was characterized by a phenotypic optimum for the two traits. When the optima strongly differ between populations (disruptive selection), we showed that the response to selection is stronger when some QTLs are pleiotropic, both at the phenotypic level (i.e. Q_{ST} values reaching higher values than without pleiotropy) and the gene level, with pleiotropic loci reaching very high F_{ST} values, close to the Q_{ST} values. In a second step, we simulated the impact of environmental change, assuming that optimal phenotype changed linearly in each population. We showed that when this change is large, it yields a large decrease in the average fitness of each population. The populations then recover their original fitness, through mutation and migration of better-adapted alleles from other populations. This recovery is, however, impossible for the most extreme scenarios of environmental change. Even when possible, recovery is very slow for strongly disruptive selection, as favourable alleles cannot be brought in by migration. F_{ST} and Q_{ST} values were also reduced during the environmental change period, as many local alleles are eliminated, while migrant alleles may be favoured by selection during this period, especially in scenarios of limited disruptive selection. The decreases in fitness and in differentiation are greater in cases of pleiotropic scenarios, as alleles favourable for both traits must be brought in by mutation or migration.

Keywords: adaptation, climate change, pleiotropy, subdivided populations

EVOLUTIONARY POTENTIAL TO FACE FUTURE CLIMATIC CHANGE OF TWO ECOLOGICALLY CONTRASTING SOUTHERN BEECHES

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The ability of populations to adapt to climate change depends on their genetic diversity as well as their plasticity. In northern Patagonia, aridity is predicted to increase due to a combination of an increase in mean temperatures and a decrease in precipitation. Rauli beech (*Nothofagus nervosa* (Phil.) Krasser) and Roble beech (*Nothofagus obliqua* (Mirb.) Blume) both occur in this area but occupy different ecological niches: the former is more tolerant of cold and shade while the latter is more resistant to drought and high temperatures. Adaptation to the new environmental conditions will only be possible if sufficient and appropriately distributed genetic diversity is available for an evolutionary response. We aimed to identify the vulnerability of the different populations in order to provide guidelines for conservation and domestication programmes. We collected 452 individuals from 13 populations along a moisture gradient (1200–3000 mm/year) and genotyped them using seven nuclear microsatellites on an ABIPRISM 3700 capillary sequencer. This showed the species to be highly differentiated (AMOVA: 0.346, $P = 0.001$), with *N. nervosa* being more diverse than *N. obliqua* (14 vs 7 private alleles, allelic richness 43.4 vs 39.8, H_o=0.396 vs 0.260). Bayesian clustering detected a maximum likelihood of four groups, with almost all (95.2 %) ind ividuals of *N. obliqua* within a single cluster. We estimated the allelic richness for each population and mapped the potential shift in their distribution with DIVA GIS under the predicted climatic conditions for 2050 under emission scenario A2 according to the average projection of three general circulation models (HadCM3, CCCma and CSIRO). *Nothofagus obliqua* showed greatest genetic diversity in eastern populations under more xeric conditions, while *N. nervosa* was more variable in western, humid locations. This confirms previous findings using chloroplast and isozyme gene markers. The results also agree with the ecological requirements of both species and suggest that conserving the current hotspots of diversity will sustain the evolutionary potential of both species, since altitudinal migration should be possible in *N. nervosa* and longitudinal migration in *N. obliqua*.

Keywords: climate change, microsatellites, *Nothofagus*, spatial genetic diversity

GENETIC IMPACT OF HUMAN LANDSCAPE MODIFICATIONS

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Human-mediated impacts on forests pose as a serious threat as the associated genetic changes could potentially have long-term evolutionary consequences. In the north-eastern United States, historical selective logging and landscape-level forest clearance have greatly shaped the structure and composition of forests, and seem likely to have genetic effects. However, these effects may be confounded by other factors such as landscape features and forest connectivity and may also be species-specific. Here, we examine the levels, patterns and magnitude of genetic diversity reduction due to human intervention in American beech (*Fagus grandifolia* Ehrh.), a wind-pollinated and disturbance-sensitive species dominant in eastern US forests. We genotyped individuals at eight highly variable microsatellite loci and compared genetic diversity between primary forests (forests that had been selectively logged but not cleared) and secondary forests (forests that naturally regenerated from abandoned agriculture lands) in Massachusetts, USA. Our general hypothesis was that secondary forests would show less genetic diversity than primary forests. Preliminary results suggest that the overall pattern of genetic diversity (measured by allelic richness, observed heterozygosity and within-population gene diversity, H_S) and genetic structure is more strongly dictated by broad physical landscape features than by human intervention. Isolated (i.e. island) and coastal populations (near range end; lower beech abundance) showed lower levels of genetic diversity (H_S 0.573; allelic richness 4.925) than inland populations (near core range of beech; H_S 0.709; allelic richness 5.802, $P<0.0005$) regardless of land-use. Furthermore, there is a significant reduction in genetic diversity and evidence of bottleneck in secondary forests compared with primary forests for those isolated and coastal populations but not for inland populations. These results suggest that isolated and peripheral populations where population sizes are small may be more vulnerable to the effects of human intervention. This has a serious implication to future forest conservation and management.

Keywords: genetic diversity, *Fagus grandifolia*, land-use

EFFECT OF THE INTERACTION BETWEEN GENE FLOW AND SELECTION ON LOCAL ADAPTATION: A CASE STUDY IN COMMON BEECH (*FAGUS SYLVATICA* L.)

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Plant species can respond to climate change by 1) migrating through seed dispersal to follow the shift in their present bioclimatic range, 2) adapting genetically to the new environment or 3) adjusting their phenotype through plasticity. Because trees are long lived and have high pollen/seed dispersal abilities, they are expected to use a combination of all of these strategies. For instance, when selection pressures are spatially heterogeneous we expect local adaptation of populations, with high level of gene flow counteracting these process by continually bringing maladapted genes. However, in the context of climate change, gene flow may bring pre-adapted genes. Populations distributed along altitudinal gradients represent a good opportunity to study the interaction between gene flow and selection in the development of local adaptation. In this study we examined the evolutionary potential of a common beech population (*Fagus sylvatica* L.) along an altitudinal gradient on Ventoux, France. The aim is to highlight genetic variations in quantitative traits potentially adaptive in the context of global warming, such as bud burst phenology, wood density and leaf morphological/physiological traits involved in carbon fixation. We present some initial *in-situ* heritability estimates based on the correlation between phenotypic traits and relatedness relationships estimated with molecular markers. We then quantified the level of contemporary gene flow within populations using parent–offspring genetic and spatial data analysis, in the frame of parentage analyses and spatially explicit mating model. This approach allowed us to estimate 1) the shape and the range of the dispersal curve for pollen and seed in common beech (mean dispersal of pollen = 42 m, mean dispersal distance of seeds = 15 m) and 2) the effect of various phenotypic traits potentially affecting male/female fecundity on relative reproductive success .Ultimately, the selection and dispersal processes experimentally characterized will be integrated in an individual-based simulation model to jointly analyse the respective roles of selection and gene flow in the evolution of quantitative traits.

Keywords: *Fagus sylvatica,* gene flow, local adaptation

CYTO-NUCLEAR INTERACTIONS IN WHITE POPLAR (*POPULUS ALBA* L.) AND EUROPEAN ASPEN (*P. TREMULA* L.) AND THEIR HYBRID

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Hybridization of species can lead to interactions between the nuclear and the cytoplasmic genome. Chloroplasts are maternally inherited in angiosperms, and chloroplast DNA gets combined with nuclear DNA of the other species in hybrid genotypes. This may affect the formation and function of photosynthetic proteins, which are encoded by both the nuclear and the chloroplast genome. Chloroplast PCR-RFLPs (cp) and nuclear microsatellites (nu) were studied in 542 individuals of 24 populations of white poplar (*Populus alba* L.) and European aspen (*P. tremula* L.) from central Europe. Based on 57 chloroplast haplotypes, two clades were detected, one for each species. Based on cpDNA within *P. alba*, clear geographic structure was found, presumably due to recolonization from disconnected glacial refugia. Conversely, in *P. tremula* cpDNA variation was more evenly distributed across the studied populations. Similarly, in nuclear DNA, 20 microsatellites (SSRs) revealed stronger geographic structuring within *P. alba* than in *P. tremula*. Hybridization was observed in both directions in zones of contact. Most hybrids appear to be F2s or subsequent generations. Significant cyto-nuclear interactions were detected within hybrids (*P. ×canescens* (Aiton) Sm.) and *P. alba* at six and four nuclear loci, respectively. Factors such as genetic drift and migration may play a role in their origin, but those are likely to affect all chromosomes. Thus, selective mechanisms are a more likely explanation for these patterns. Linkage of microsatellite markers to certain genes involved in cyto-nuclear processes might cause the observed disequilibria, especially in early generation hybrids carrying relatively large chromosome blocks inherited from each parental species. The implications of our findings and opportunities for future work will be discussed.

Keywords: admixture zones, cyto-nuclear disequilibrium, hybridisation, poplars

LONG HISTORY AND QUATERNARY-RANGE DYNAMICS IN THE GENUS *MYRTUS* AROUND AND BEYOND THE MEDITERRANEAN BASIN

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The Myrtaceae family encompasses more than 4000 tropical species, but only the genus *Myrtus* is distributed in the northern hemisphere. The common myrtle (*Myrtus communis* L.) is an emblematic evergreen shrub of the thermophilous Mediterranean flora, whereas the Nivelle myrtle (*Myrtus nivellei* Batt. & Trab.) is a centro-Saharan endemic species with a very disjunct distribution in extremely harsh conditions above 1500m (Algeria: Hoggar, Tassili n'Ajjer, Tassili n'Immidir and Tefedest; Chad: Tibesti). As the genus *Myrtus* has persisted since the Tertiary period, it may reflect the evolutionary response of flora to climatic change during the Quaternary period and the evolutionary footprint of the complex history of Mediterranean ecosystems. Chloroplast and ribosomal DNA intergenic spacers (*trnL-F, EucpsbA-Eurorpl2*, ETS and ITS) were sequenced in 172 populations of *M. communis* around the Mediterranean sea and 23 populations of *M. nivellei* from mountains in the Sahara. Genetic structure analyses reveal higher genetic distances within some populations of *M. communis* than between *M. communis* and *M. nivellei*. Both network nested analysis of cpDNA markers and spatial principal component analysis of rDNA markers showed strong east–west genetic differences, resulting in a bipolarization of the genetic diversity around the Mediterranean basin. Analysis of genetic diversity indicates long-term persistence of *M. communis* on both sides of the Mediterranean basin, but also a more recent range expansion, probably linked to the shrub's putative long-distance dispersal strategy. Surprisingly, *M. nivellei* appears to originate from various Mediterranean lineages of *M. communis*, with at least three migration events. Genetic diversity parameters calculated for *M. nivellei* indicate a relatively recent isolation, probably linked to the succession of wet and arid phases in the region during the Quaternary.

Keywords: genetic diversity, Mediterranean, migration, *Myrtus*, phylogeography, Sahara

GENETIC VARIATION AND PLASTICITY OF DROUGHT REACTION IN NORWAY SPRUCE (*PICEA ABIES* (L.) H. KARST.) POPULATIONS AS REVEALED BY X-RAY DENSITOMETRIC MEASURES OF WOOD DENSITY

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In lowland forests of eastern Austria high temperatures, limited precipitation (e.g. in Vienna: precipitation sum May to August is 278 mm, mean air temperature from May to August is 17.8°C) and the occurrence of droughts limit tree growth, in particular that of Norway spruce (*Picea abies* (L.) H. Karst.). An increase in the frequency of temperature-induced droughts, expected due to climate change, seems to worsen this situation. We analysed tree cores from three provenance experiments with Norway spruce located in eastern Austria to identify drought-resistant provenances and drought-resistance genes. X-ray densitometry has often been used to investigate the relationship between maximum density and summer temperature at temperature-limited sites, and we used it to screen 1000 cores from trees of about 35 years old of 17 provenances. Trees from the most drought-affected trials (Porrau and Deutsch-Gerisdorf) showed significant growth reductions during drought events in 1992 and 2003. We characterized the effect of drought on various wood density and increment measures and analysed the genetic variation among the provenances. The plasticity of drought response of the provenances was analysed by comparing the responses of provenances in various trials and drought periods. The significant differences observed among provenances provide a basis for further provenance selection and for the setting up of collections of individuals with more or less drought sensitivity for genomic analysis.

Keywords: drought resistance, genetic variation, *Picea abies*, plasticity, x-ray densitometry

Session H:
Migration under climate change

POLLEN DISPERSAL IN A SELF-INCOMPATIBLE SPECIES, THE WILD CHERRY (*PRUNUS AVIUM* L.)

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Pollen-mediated gene flow is a major component of gene flow in several tree species. Estimates of pollen dispersal have usually revealed considerable long-distance dispersal of pollen. However, the effects of such important traits as mating systems and sexual polymorphisms on patterns of pollen dispersal and male mating success are largely unknown. The general aim of the present study was to describe patterns of pollen dispersal and male mating success (MMS) in a scattered forest tree species, wild cherry (*Prunus avium* L.). The sexual reproduction of the species is controlled by a gametophytic self-incompatibility (GSI) system. Genes controlling the system are known and have been sequenced. This species also often reproduces through resprouting, leading to clusters of incompatible stems. The GSI system is thus expected to have a significant effect on MMS, at least comparable to that of distance or any component of the phenotype. Consequently, we introduced this reproductive character in a spatially explicit mating model. We studied two contrasting populations, one large and continuous, the other small and fragmented. Our study showed that modelling mating system in a GSI species improved MMS predictions; mating system was the main factor explaining variations of MMS in the small population. Our study also showed that pollen dispersal distances are underestimated when GSI is not included in the model as GSI limits mating at short distances. The implications of our results are discussed in the context of environmental change.

Keywords: gametophytic self-incompatibility, pollen dispersal, population size, *Prunus avium*, reproduction system

POPULATION DIFFERENTIATION OF SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL.) AT THE ALTITUDINAL FRONT OF MIGRATION IN THE FRENCH PYRENEES

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To assess the effects of altitude on genetic diversity, a genetic survey was conducted in 12 populations of sessile oak (*Quercus petraea* (Matt.) Liebl.) located between 130 m and 1660 m above sea level in two parallel valleys on the northern side of the Pyrenees mountains. Genetic diversity was monitored with 16 nuclear microsatellite loci and 5 chloroplast markers. The cpDNA survey suggested that extant populations in both valleys shared the same source populations from the plain. There was no visible trend of nuclear genetic diversity along the altitude gradient, although indirect estimates of effective population sizes revealed a consistent reduction at higher altitudes. Population differentiation, although low, was mostly present among populations within a valley and reached similar levels to differentiation across the range of distribution of sessile oak. Overall differentiation in the valleys was mostly due to the genetic divergence of the highest populations and the clinal altitudinal variation of allelic frequencies at a few loci. Bayesian analysis of migration rates between pairs of populations showed that gene flow is preferentially unidirectional from lower to higher altitudes. Clonality was observed in high-altitude populations. Introgression of pedunculate oak (*Q. robur*) and downy oak (*Q. pubescens*) was more frequent at the altitudinal margin, suggesting that this mechanism may contribute to the migration and adaptation of *Q. petraea* under these very constrained environmental conditions.

Keywords: altitudinal gradient, gene flow, genetic diversity, hybridization, *Quercus*

POPULATION STRUCTURE AND GENETIC DIVERSITY OF SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) ASSESSED BY MICROSATELLITE MARKERS

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Sweet chestnut (*Castanea sativa* Mill.) is the only species of the genus *Castanea* in Europe. Its population structure appears to be the result of the combined effects of climate oscillations, artificial selection and local effects of isolation by distance. Previous studies based on genetic analysis and palynological data indicated the likely centre of origin of *Castanea sativa* to be in north-east Turkey and the Caucasus. In this research we carried out the first large-scale genetic analysis of chestnut populations using microsatellite markers to obtain accurate information on its area of origin and its migration pattern. The two main objectives of our work were to: 1) confirm the origin of sweet chestnut in Turkey; and 2) to assess the genetic structure of European populations. The population structure and genetic diversity of 778 individuals representative of 31 European populations of *C. sativa* were analysed using six microsatellite markers. Genetic structure was investigated using multivariate analysis and the Bayesian clustering algorithm implemented in the STRUCTURE v. 2.2 software. The Turkish populations showed higher F_{IS}, F_T and F_{ST} than the other European populations. Distinct gene pools were observed: one covering Italy and Spain, one located in eastern Turkey and one covering populations in Greece and western Turkey. The reduction of genetic diversity from east to west combined with the observed structure of the populations indicates the east Turkey area as the possible centre of origin of the species. The results reported in our work establish the genetic baseline required for further investigations aimed at defining more appropriate strategies for conservation and sustainable management of chestnut genetic resources.

Keywords: *Castanea sativa*, centre of origin, migration pattern, population structure

THE DISTRIBUTION PATTERN OF MITOCHONDRIAL DNA (MTDNA) OF THE SILVER FIR (*ABIES ALBA* MILL.) IN THE WESTERN BALKANS

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A study of silver fir (*Abies alba* Mill.) using mitochondrial DNA (mtDNA) markers identified two variants of the *nad*5 gene and revealed strong east–west differences in populations. Populations from the western part of the range exclusively contained one variant while populations from the eastern part of the range contained only the second variant. Mixed populations were observed in Croatia, Slovenia and north Italy. A study of mtDNA haplotypes in the Ukranian Carpathians revealed a sharp boundary between the ranges of the two types. The situation in the western Balkans was more complicated and two migration streams in opposite directions were proposed to explain the observed pattern. The aim of this study was to obtain a more detailed determination of haplotype division of silver fir in Croatia, Bosnia and Herzegovina, Slovenia and Albania. Except major populations from Croatia, the investigation focused on small, isolated populations from the sub-Mediterranean area of Bosnia and Herzegovina, in order to determine their relationship to the populations from the central parts of their natural distribution in Bosnia and Herzegovina and central Europe. Samples for DNA extraction were taken from a provenance trial that included 18 Croatian and 2 Slovenian provenances, and from 7 natural populations in Croatia, 8 populations in Bosnia and Herzegovina and two populations in Albania. The DNA fragment (*nad*5-4) used in previous studies was used also for this study. Thirteen populations contained only the longer allele (allele 1) that is typical of central and western European populations. The shorter allele (allele 2), typical of south-eastern European populations, was found in 16 populations. Nine populations had mixed haplotypes. Two populations from Albania contained only allele 2. To the south and south-east of the mountains of Čabulja, Šator, Grmeč and Oštrelj in Bosnia and Herzegovina, only allele 2 was found. Therefore, this study reveals, similarly to a previous study on silver fir looking at mitochondrial DNA, two parallel clines of mixed haploid types from north-eastern Italy, across Slovenia and Croatia, to the western part of Bosnia.

Keywords: haplotypes, postglacial migration, suture zone, variation

THE IMPACT OF THE CONTACT ZONE BETWEEN TWO MIGRATION ROUTES OF SILVER FIR (*ABIES ALBA* MILL.) IN THE WESTERN BALKAN AREA

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Silver fir (*Abies alba* Mill.) is one of the most important forest tree species in the western Balkan area both economically and ecologically. Its distribution has been the subject of scientific research for many years because silver fir from these areas displays very interesting morphological and genetic differentiation. The objective of this research was to determine the boundary of gene introgression of silver fir deriving from the Apennine and Balkan glacial refugia in the western Balkan populations with the use of isoenzymes. Nine enzyme systems were studied in 24 populations from Bosnia and Herzegovina and Croatia, and polymorphic gene loci and their allelic types were scored. The analysis of general genetic parameters did not allow for any conclusion, and neither did the analysis of genetic distances. However, statistical analysis of allele distribution showed the populations from Croatia (Gerovo, Fužine, Skrad and Vrbovsko) to be distinctly different from those from Bosnia and Herzegovina. Populations along the contact zone (Grmeč, Oštrelj, Tičevo and Glamoč) and from the eastern part (from central and east Bosnia and Herzegovina) did not show any regular grouping or clinal variability. This is attributed to high ecological diversity, fragmentation and the occurrence of genetic drift. Alleles originating from the Apennine refugium are also present in trees in eastern Bosnia. The results suggest that silver fir originating from the Balkan refugium was more widely distributed in the past, but is now retreating relative to that originating from the Apennines.

Keywords: *Abies alba*, Apennines, migration route, western Balkans

FLOWERING PHENOLOGY AS AN ESSENTIAL PARAMETER FOR POLLEN FLOW MODELLING IN BLACK POPLAR (*POPULUS NIGRA* L.)

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European black poplar (*Populus nigra* L.) can be found in three interconnected ecosystems: 1) natural riparian forests; 2) cultivated stands through *P.* ×*euramericana* interspecific hybrids; and 3) ornamental plantings through *P. nigra* cv 'Italica' (i.e., Lombardy poplar). As a first step to assess the potential impact of cultivated and ornamental poplars on the genetic diversity of wild populations, pollen flow was modelled within a wild *P. nigra* stand located close to a Lombardy poplar row. A total of 484 wild *P. nigra* adult trees were inventoried on the 11.5 ha study site located along the Loire River (EVOLTREE ISS Loire – zone 4). Several physical and biological parameters, such as flowering phenology, physical distance and tree size, were measured on all trees. Pollen flow was estimated by collecting 1680 seeds from 31 females distributed over the site, and by modelling pollen dispersal using mating model and paternity analysis based on 10 SSR markers. We detected a high immigration rate (45%), and 4% of the seeds could be attributed to Lombardy poplars. The model that best fitted the data involved an exponential power distribution curve with a fat-tailed dispersal kernel. Phenological assortative mating and short-distance pollen dispersal were identified as the main factors influencing reproductive success and hybridization between Lombardy and wild poplars.

Keywords: flowering phenology, mating model, pollen flow, *Populus nigra*

IS THERE A PLEISTOCENE OR HOLOCENE REFUGIUM FOR THE HYBRID ZONE BETWEEN EUROPEAN ASH (*FRAXINUS EXCELSIOR* L.) AND NARROW-LEAFED ASH (*FRAXINUS ANGUSTIFOLIA* VAHL)?

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In order to understand the origin of environmentally driven hybrid zones one must know whether present-day zones of hybridization have been stable over time. In particular, exploring the effects of major climate regimes, such as those existing during the last glacial maximum (21 000 years ago) and the Holocene optimum (6000 years ago), on the probable distribution of hybrid zones may give insights into the present-day genetic structure of parental populations. Moreover, the identification of areas that may have contained hybrid zones during the Holocene optimum (2°C warmer than at present) may help to design strategies for genetic resources management with respect to climate change. One such case is the hybrid zone between the close species *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl (Oleaceae) that presently occurs within a gradient determined by summer precipitation and winter frost levels in intermediate regions such as the Loire and Saône valleys in France. We used species distribution models based on maximum entropy (MaxEnt) and state of the art paleoclimate models (CCSM3, FGOALS-g.1.0, IPSL-CM4-V1-MR and MIROC3.2) at 21 000 and 6000 years before present to simulate multimodel consensus distributions of both species and their hybrid zones. Results of the simulations suggest that both species shared the same well-known refugia (Iberian and Italian peninsulas, the Adriatic coast, the Balkans, southern coast of the Black Sea and north-western Africa). However, the highest probability for each species did not necessarily coincide, suggesting different allopatric microrefugia. No evidence for Pleistocene hybrid refugia was found. In contrast, simulations showed that climates compatible with present hybrid zones were widely distributed during the Holocene, especially along the Atlantic coast of western Europe. Overall, results suggest that the warmer conditions of the Holocene may have promoted hybridization between these two close species, while hybrid zones during the cold periods of the Pleistocene seem less likely. Taken altogether, the simulations suggest that present-day environmentally driven hybrid zones are susceptible to modification by variable climate conditions, an issue that is worth examining in detail considering the rapid climate change that it is being experienced now.

Keywords: climate change, *Fraxinus angustifolia*, *Fraxinus excelsior*, Holocene, hybridization zones, Pleistocene

CONTEMPORARY SEED AND POLLEN DISPERSAL ABILITIES OF SILVER FIR (*ABIES ALBA* MILL.) AT RANGE MARGINS

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Effective gene flow from parent to offspring is a major process shaping plant population genetic diversity and evolutionary potential. However, little is still known about the extent of within-species variation in contemporary patterns of pollen and seed dispersal, and even less on ecological drivers shaping this variation. Silver fir (*Abies alba* Mill.) is a keystone tree species in low to mid-altitude European mountainous forest ecosystems, and appears to be particularly sensitive to ongoing climatic change. In this study, we investigated contemporary patterns of gene flow at southern and northern margins of the European distribution range of silver fir, using two populations in a Mediterranean mountainous forest (EVOLTREE ISS Ventoux, France) and two populations in an old-growth forest (EVOLTREE ISS Blyzin, Poland). We used spatially explicit parentage models and a combination of nuclear and chloroplast DNA microsatellite markers to estimate seed and pollen dispersal kernels in naturally established seedlings. Patterns of contemporary gene flow appeared to vary across populations. Selfing rates were higher in southern populations (average $s = 0.18$) as than in northern populations (average $s = 0.07$). Pollen dispersal also differed strongly among sites, with fat-tailed dispersal kernels and average pollen dispersal distance (δ_p) >100 m in northern populations, while southern populations showed exponential pollen dispersal kernels and restricted pollen dispersal distance (δ_p <11 m). Patterns of seed dispersal were less variable among populations, with a general tendency for exponential dispersal kernel and average seed dispersal distance ranging between 18.2 and 24.5 m. Both pollen and seed immigration rates were highly sensitive to assumptions on background pollen pool frequencies, due to low resolution of the marker set. These results indicate limited pollen production and dispersal abilities in trailing-edge populations as compared with leading-edge populations, as well as overall limited seed dispersal abilities relative to rates of ongoing and predicted climate change.

Keywords: contemporary gene flow, microsatellite, parentage analysis, pollen dispersal, seed dispersal, selfing

PATERNAL INTROGRESSION FROM SIBERIAN SPRUCE (*PICEA OBOVATA* LEBED.) TO NORWAY SPRUCE (*P. ABIES* (L.) H.KARST.): TRACING POLLEN AND SEED FLOW WITH CHLOROPLAST AND MITOCHONDRIAL DNA

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Norway spruce (*Picea abies* (L.) H. Karst.) and Siberian spruce (*Picea obovata* Lebed.) are closely related and occupy a large parapatric area across the whole of northern Eurasia. It has been suggested that a wide zone of hybridization along the Ural Mountains separates the European Norway spruce from the Siberian spruce. In this study we analysed maternally (mitochondrial DNA, mtDNA) and paternally (chloroplast DNA, cpDNA) inherited markers in 50 spruce populations to study genetic differentiation and introgressive hybridization between them. We used partial sequences of the cpDNA *trn*T-*trn*F region and size variants of the mtDNA *nad1* region, the latter by extending a previous data set. Fourteen mtDNA and four cpDNA haplotypes were identified, and distinct genetic division was detected between Norway spruce and Siberian spruce. In the mtDNA marker, a 9-bp polymorphism differentiated populations in the northern Urals and east Siberia from populations along and west of the Ural Mountains. This division largely corresponded to two highly differentiated genetic (SAMOVA) groups both in the mtDNA dataset ($F_{CT} = 0.685$) and in the cpDNA dataset ($F_{CT} = 0.438$), suggesting that the main border between the taxa is to the east of the Ural Mountains. However, the paternally inherited cpDNA marker showed extensive introgression from Siberian spruce into the entire range of Norway spruce, and the maternally inherited mtDNA haplotypes of Norway spruce are also found across the Ural Mountains. Introgression via pollen thus acts as a mechanism of dispersal of Siberian spruce genes into the northern European gene pool of Norway spruce. No phylogeographic structure was detected in Siberia, consistent with a single glacial refugium for Siberian spruce, but only little variation was detected with the markers surveyed here.

Keywords: introgression, pollen, *Picea abies*, *Picea obovata*, spruce

SPECIES DISTRIBUTION SHIFTS AND EFFECTS OF FORECASTED CLIMATE CHANGE ON TREE PHENOLOGY OF EUROPEAN TREE SPECIES

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Species distribution ranges are strongly affected by climate change. Numerous studies based on ecological niche models conclude that species will shift northward and/or upward in response to warming climate, but few observational studies have assessed this hypothesis, especially over a large scale. Moreover, modelling phenology is crucial to assess the impact of climate change on canopy duration and productivity of terrestrial ecosystems. Focusing on dominant tree species in Europe, the aims of this study were to: 1) examine potential latitudinal and altitudinal shifts in species occurrence; 2) examine the accuracy of various leaf phenology models; 3) assess the current and the future role of chilling and forcing temperatures on flushing; and 4) predict seasonal shifts in response to climate warming. Our analyses were based on data from national forest inventories and long-term phenological monitoring along altitudinal gradients. Our study showed northward and upward shifts in occurrence of temperate species, as suggested by niche models. However, such tree species' migration is much slower than that proposed by models. The single-phase models (based solely on forcing temperatures) were as efficient as two-phase models (based on both chilling and forcing temperatures) for most species, suggesting that chilling temperatures are currently sufficient to fully release bud dormancy. However, our predictions for the 21st century highlight that chilling temperature could be insufficient for some species at low altitude. Overall, flushing is expected to occur earlier in the coming decades but this trend differed dramatically between species (from 0 to 2.4 days per decade). Prediction of leaf senescence is more challenging; the models work properly for only two deciduous species (oak and beech), for which senescence is expected to be delayed in the future (1.9 days per decade). Simulations over the 21st century predict a greater lengthening of canopy duration for oak than for beech, suggesting that the competitive balance between species could change as climate warms, and shifts in their altitudinal distributions might occur.

Keywords: competitive balance, growing season, modelling, phenology, species migration

Open poster session

FOREST TREE GENOMICS IN DEVELOPING COUNTRIES: A CASE FOR SUB-SAHARAN AFRICA

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There is great potential for more rapid tree improvement by applying new breeding strategies based on progress in genomics. However, application of genomics is still very limited in developing countries, especially in sub-Saharan Africa. In this region, forest cover continues to decline at an alarming rate as forests are cleared for settlement and agricultural expansion and forest resources are over-exploited. This has resulted in loss of important germplasm resources, many of which are endemic to the region. The situation is aggravated by unpredictable weather and increasing drought as a result of climate change, which makes the process of re-establishing forests even harder due to reduced seedling survival and high tree mortality rates. The application of cutting-edge tools of genome analysis is critical in the sustainable management of forest trees, as demonstrated by several tree genome analysis programmes in developed countries. Knowledge obtained guides efforts to improve trees for desired end-uses or enhancing the ability of trees to adapt to environmental stresses such as pollution and climate change. This paper highlights some of the factors restricting tree genomic research in sub-Saharan Africa and explores possible interventions that will see increased tree genomic activities in the region.

Keywords: genomics, sub-Saharan Africa, tropical trees

ECOLOGICAL GENETICS INVESTIGATIONS OF DIPTEROCARPS WITH DIFFERENT LIFE HISTORY TRAITS

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Forest genetic resources conservation programmes must be guided by the biological attributes of the target species. We cannot conserve effectively what we do not understand. What constitutes the most critical biological information for plant conservation has been discussed for the past 30 years. Most scientists promote an approach that is either ecological or genetic in emphasis. Ecological and genetic processes often interact synergistically to influence population viability and to determine the long-term persistence of populations. Consequently, conservation management of plant species requires both ecological information and a robust understanding of underlying genetic processes and variation within and between populations. Tropical forests are rich in plant species diversity; for the majority of these species, obtaining adequate knowledge for setting up conservation strategies is impossible. It is suggested that tree species can be grouped according to their life history traits. The information generated for one species then can be adapted for other species that have similar type of life history traits. Accordingly, we have recently completed a series of ecological genetics investigations to develop conservation strategies for the following species: 1) *Shorea lumutensis* (rare endemic and predominantly outcrossing dipterocarp); 2) *Neobalanocarpus heimii* (widespread endemic and predominantly outcrossing dipterocarp); 3) *Hopea bilitonensis* (rare and predominantly selfing dipterocarp); and 4) *H. subalata* (rare endemic dipterocarp with apomictic mode of reproduction). These allowed the prediction of optimum population sizes for effective conservation and sustainable utilization of tropical plant species with similar life history traits.

Keywords: conservation management, Dipterocarps, ecological genetics, life history traits

DEVELOPMENT OF A NOVEL SET OF EST-SSR IN TAMARISK (*TAMARIX* SPP.) AND CHARACTERIZATION OF THREE POPULATIONS IN SOUTHERN ITALY

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Tamarisk (*Tamarix* spp.) plants are resistant to abiotic stresses, as they thrive in zones where drought, soil salinity and high temperature are common. Their survival capacity and genetic diversity can be harnessed to help sequester atmospheric CO_2 by planting tamarisk in marginal areas. Italian coastal zones could provide *Tamarix* germplasm adapted to hot, dry and saline habitats, but there is no information about the genetic structure of tamarisk populations in Italy. Few molecular markers have been developed for *Tamarix*, but expressed sequence tags (ESTs) show promise as markers. The aim of this work was to develop a novel set of polymorphic EST-SSRs in *Tamarix* to be used as an additional tool for taxonomy studies and for the management of natural resources or breeding programmes. Eight polymorphic EST-SSRs were selected and analysed in 163 plants from three populations from southern Italy: Basento River (Basilicata), Simeto River (Sicily) and Crati River (Calabria). Each site is characterized by a wide variety of environments, and plant material was collected along altitudinal, water availability and salinity gradients. Forty-nine plants were identified with Baum's morphological keys as belonging to African tamarisk (*T. africana* Poir.), French tamarisk (*T. gallica* L.) and Canary Island tamarisk (*T. canariensis* Willd.) but the remainder remained unidentified. The set of EST-SSRs and a Bayesian assignment method were used for species identification, establishing the correspondence with the previously described taxa of our unidentified individuals. EST-SSR markers showed cross-species amplification, and intraspecific and interspecific polymorphism were observed. Our results provide a first contribution to identifying the genetic diversity of Italian *Tamarix* species. The amount and distribution of genetic variability of *Tamarix* plants found will clarify their ecological role and offer information about genetic basis of abiotic stress tolerance in *Tamarix*.

Keywords: abiotic stress, EST-SSR, genetic variability, *Tamarix*

TREE RESPONSES TO FRAGMENTATION: THE CASE OF THE THREATENED COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) IN NORTH-WEST EUROPE

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In general, habitat fragmentation decreases the genetic diversity of plant populations. For trees, the genetic effects of fragmentation are less clear as they generally show extensive gene flow. We investigated genetic effects of fragmentation on a threatened tree, common juniper (*Juniperus communis* L.), in a highly fragmented landscape in north-west Europe. We analysed patterns of genetic variation on two spatial scales by using AFLP and microsatellite markers and investigated whether genetic diversity was correlated with population size, age structure and seed viability. Although common juniper has a limited potential for gene flow, we found no clear negative effects of fragmentation on genetic diversity. We explain this apparent paradox by the long life span of the tree combined with the relatively recent population fragmentation and an underlying highly diverse, continuous population prior to fragmentation. Our results suggest that, to date, inbreeding depression is not the major risk factor for juniper populations in north-western Europe and likely not the underlying cause of the low seed viability observed. This study shows that the genetic effects of fragmentation may be slow to appear in trees. Our data also support the idea that during the last glacial maximum trees were probably much more widespread in northern Europe than hitherto thought. The processes behind the low seed viability need to be clarified by further research. In the meantime, conservation strategies should focus on establishment of gene banks, preventing further habitat degradation and rejuvenating populations by planting with local material.

Keywords: ALFP, common juniper, genetic differentiation, habitat fragmentation, inbreeding

DESPITE SMALL HABITAT SIZE AND ISOLATION, SCOTS PINE (*PINUS SYLVESTRIS* L.) POPULATIONS OF EAST-CENTRAL EUROPE HAVE MAINTAINED HIGH LEVEL OF CHLOROPLAST HAPLOTYPIC VARIATION

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The distribution of Scots pine (*Pinus sylvestris* L.) is highly fragmented across east-central Europe. Small, peripheral populations occur in a wide range of environments. In the present study we investigate the genetic structure and diversity of the native *Pinus sylvestris* populations by using chloroplast microsatellite markers. Five isolated populations from east-central Europe were sampled from different habitat types (mountain raised bog, mountain rocky outcrop and lowland sandy area) and compared with three provenances from the northern part of the species' European range. The three chloroplast microsatellites revealed high levels of haplotypic variation in each population. In spite of their limited size, the peripheral populations also displayed high variation, with the most variable ones found on the raised bogs of the eastern Carpathians. AMOVA analysis revealed low genetic structuring of populations, with only 5.64% of the total variation derived from population differentiation. As expected, the most divergent population, which grouped separately in SAMOVA analysis, was that from the lowland sandy outcrop in Hungary. Populations from wet habitats clustered together with the Nordic populations.

Keywords: chloroplast microsatellite, haplotype, peripheral population, *Pinus sylvestris*

QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO PATHOGENS IN PEDUNCULATE OAK (*QUERCUS ROBUR* L.)

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Powdery mildew, caused by *Erysiphe alphitoides* (Ascomycete) is the most frequent disease of oaks, which are also known to be host plants for *Phytophthora cinnamomi* (Oomycete), the causal agent of ink disease. Components of genetic resistance to these two pathogens, infecting either leaves or root and collar, were investigated in a full-sib family of pedunculate oak (*Quercus robur* L.) that was vegetatively propagated by cuttings. Resistance to powdery mildew was assessed by two methods. First, inoculations of *E. alphitoides* were performed under controlled conditions on excised leaves removed from cuttings grown in the greenhouse. The level of host–pathogen compatibility was assessed by recording infection success and mycelial growth. Second, the progeny were assessed for susceptibility to powdery mildew in the field under natural infection conditions over three years. Resistance to ink disease was estimated by inoculating *P. cinnamomi* on stems on two-year-old cuttings (one cutting per genotype) grown in a glasshouse and by measuring the length of the induced lesion in two experiments. Preliminary results showed that quantitative trait loci (QTLs) associated with the response to these pathogens were located in 13 genomic regions of the genetic linkage maps available for the two parents. Their effects explained between 4% to 55% of the phenotypic variation. However we could not identify co-localization between major QTLs involved in both diseases. Although the genetic architecture of resistance to *E. alphitoides* varied between years and infection conditions, stable QTLs were detected. Given that infection by this fungus is strongly dependent on the phenological status of its host, co-localizations between QTLs for resistance and those for phenology were investigated.

Keywords: disease resistance, oak, *Phytophthora*, powdery mildew

CONSERVATION AND DIVERSITY OF *POPULUS* SPECIES IN CASTILLA Y LEÓN, SPAIN

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Populus species native to Spain, aspen (*P. tremula* L.), white poplar (*P. alba* L.) and gray poplar (*P.* × *canescens* (Aiton) Sm.), are a valuable genetic resource for the forestry sector. However, their populations are rapidly and widely disappearing. Their distribution in marginal ecological niches, limited abilities to colonize new areas and the immediate effects of climate change affect their long-term survival. *Ex situ* conservation is important to ensure conservation of a broad genetic base to provide materials adapted to a wide range of environmental conditions. Information on the current status of groves, their geographical distribution and existing data on genetic variability reflect a different scene in the species studied. Exhaustive sampling in the community of Castilla y León, Spain, showed concentric distribution of the three species. Aspen is found in small scattered populations on the mountains that surround the community. White poplar is found on the interior plain, and gray poplar is found in the intermediate zone, between the mountains and the plain. Diversity assessment using microsatellites and isozymes indicated high genetic diversity within and between populations of aspen, whereas in gray and white poplars there are large populations consisting of a single clone. Clones of all three species showed marked shortage of female trees. Such information is essential to propose conservation strategies. In this communication we present and discuss the development of *ex situ* genetic resources collections and more-efficient sampling strategies for each of the species.

Keywords: *ex situ* conservation, genetic diversity, geographic distribution, *Populus*

INFLUENCE OF POPULATION SIZE AND SPATIAL STRUCTURE ON GENETIC DIVERSITY OF IBERIAN PINES

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Habitat fragmentation reduces population size and increases spatial isolation, which is expected to lead to a decrease in genetic variation and potentially an increase in genetic divergence among populations due to reduced gene flow. The response to habitat fragmentation is probably also dependent on species life history. To examine this hypothesis we analysed a set of chloroplast microsatellites and isoenzymes in Iberian pine populations to investigate levels of genetic diversity, the distribution of genetic diversity within and among populations (population structure) and the potential consequences of fragmentation on genetic diversity. The study covered all six pine species found in the Iberian Peninsula: Aleppo pine (*Pinus halepensis* Mill.), European black pine (*P. nigra* Arn.), maritime pine (*P. pinaster* Ait.), mountain pine (*P. uncinata* Ram.), Scots pine (*P. sylvestris* L.) and stone pine (*P. pinea* L.). Genetic variation was assessed using a set of common chloroplast microsatellites (105 populations analysed) and isoenzymes (64 populations of *Pinus halepensis*, *P. nigra*, *P. pinea* and *P. pinaster*). We investigated the effects of habitat fragmentation on each species by looking at the relationship between standard neutral genetic variability parameters and specific population parameters, particularly population size, fragmentation and isolation indices calculated using GIS software. We discuss some genetic effects that reduction and restructuring of populations could have on Iberian pine species.

Keywords: genetic diversity, fragmentation, *Pinus*

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