Population Genetics and Genomics of Forest Trees: from Gene Function to Evolutionary Dynamics and Conservation

October 1st – 6th, 2006
Alcalá de Henares, Madrid. Spain

Organizing Institutions:

Universidad de Alcalá
INIA Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
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SCIENTIFIC PROGRAMME AND ABSTRACTS

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SUMMARY

INTRODUCTION

SCIENTIFIC PROGRAMME

ABSTRACTS

INTRODUCTION

Forest genetics has experienced a significant revolution in recent years. The development of forest genomics and high-throughput genotyping methods, together with new and refined statistical approaches for population analysis, have brought extraordinary advances in the study of evolutionary processes in the wild. Tree species, such as pine, spruce, poplar and eucalyptus, are nowadays considered non-classical models for population, evolutionary and ecological studies. Genetic diversity of forest trees is recognised to have ecosystem-wide importance. Multidisciplinary approaches, bringing together ecologists, geneticists and physiologists, amongst others, are now combined to understand the genetic determinism of adaptive traits and to study the patterns of adaptive variation at landscape level.

In this spirit, an IUFRO Conference will be held in the exceptional venue of Alcalá de Henares (Madrid, Spain), in the line with previous IUFRO Working Group meetings on Population, ecological and conservation genetics (2.04.01) and Genomics (2.04.10). The IUFRO Conference will be organised jointly with the EU COST Action E-28 final meeting (Genosilva: European Forest Genomics Network), effectively merging genomics and population and ecological genetic approaches. A selection of invited speakers from different disciplines and the organization of thematic workshops will contribute to cutting-edge discussions, while enjoying the charming environment of the town of Alcalá de Henares. Multiple manifestations of Spanish history, from Romans to the Renaissance, are found in its churches, monuments and charming streets, which witnessed the birth of Miguel de Cervantes in 1547. The University, one of the oldest in Spain, became a model for other centres of learning in Europe and America after its foundation in 1499, which gave way to a process of urban renewal that transformed Alcalá into a university city built in accordance with the canons of classical architecture. UNESCO recognised the city and the University of Alcalá as a World Heritage Site in 1998.

SCIENTIFIC PROGRAMME

IUFRO PROGRAMME

Sunday, October 1st

18.00	Conference check-in
19.00	Welcome cocktail

Monday, October 2th

8.00 – 9.15	Conference Opening. Dr. Giuseppe G. Vendramin (CNR, Italy
9.15 – 10.00	Dr. Philip W. Hedrick (Arizona State University, USA Recent developments in conservation genetics
10.00 – 10.30	COFFEE BREAK

Session I
Gene diversity, phylogeography and population genomics

Chairs: O.Savolainen and G.G. Vendramin

10.30 – 10.45	Cervera, María Teresa Clinal patterns of nucleotide diversity and differentiation at candidate genes for drought stress tolerance and bud phenology in a maritime pine metap
10.45 - 11.00	Eveno, Emmanuelle Molecular genetic differentiation at candidate genes for drought stress tolerance and microsatellite markers in Pinus pinaster natural populations
11.00 – 11.15	Ishiyama, Hiroko The demographic history of Shorea parvifolia (Dipterocarpaceae) inferred from nucleotide polymorphism at multiple nuclear genes
11.15 – 11.30	Namroud, Marie-Claire A SNP genome scan for the search of gene polymorphisms involved in adaptive population differentiation in white spruce
11.30 - 11.45	Schroeder, Hilke Genetic variation and differentiation of the green oak leaf roller (T. viridana) and its host (Quercus robur)

11.45 - 12.00	Szmidt, Alfred E. Pattern of nucleotide polymorphism in the gMdh nuclear gene region of Eurasian Larix (Pinacea) specied
12.00 - 12.15	Vendramin, Giovanni Genetic diversity and linkage disequilibrium in drought tolerance candidate genes in Aleppo pine (Pinus halepensis Mill)
12.30 - 14.00	LUNCH
14.00 - 14.30	Presentation of the “Marcus Wallenberg Prize 2006”

Session II
Conservation Genetics

Chairs: P.Hedrick and D.Boshier

14.30 – 14.45	Chybicki, Igor Genetic processes during natural regeneration within the mixed oak stands
14.45 – 15.00	Fernández Carrillo, Laura Genetic diversity in mediterranean forest patches: A case study
15.00 – 15.15	Fernández, Jesús Estimating heritability from molecular marker information: the accuracy of a regression approach
15.15 – 15.30	Heuertz, Myriam Phylogeography of African lowland rainforest trees: preliminary results
15.30 – 15.45	Jaramillo-Correa, Juan Pablo Imprints of the Holocene collapse in the modern cytoplasmic DNA population structure of threatened montane conifers from northern Mexico
15.45 – 16.00	Larsen, Anders Søndergaard Genetic studies of native Malus sylvestris populations in Denmark: is the local gene pool endangered?
16.00 – 16.30	COFFEE BREAK

Session II (cont.)

16.30 – 16.45	Nettel, Alejandro Extinction, trans-oceanic dispersal, and gene flow in a tropical wetland forest species
16.45 – 17.00	Sánchez, Leopoldo Heritabilities out of the beaten test site: looking at the perspectives of in situ inferences for forest tree species with a preliminary study in Quer
17.00 – 17.15	Sutherland, Bruce Molecular diversity and population structure in native ash (Fraxinus excelsior) populations in the United Kingdom
17.15 – 18.00	Presentation of EVOLTREE NoE (Dr. Antoine Kremer, INRA, France
18.00	Poster Session I

Tuesday, October 3rd

8.00 – 9.15	Dr. Christian Lexer (Royal Botanic Gardens, Kew, UK) - (Post-) genomic studies of species barriers: from annual plants to forest trees

Session III
Gene flow, spatial structure and hybridization

Chairs: O.J. Hardy and P.E. Smouse

9.15 – 9.30	Boshier, David Pollen flow and siring success in mast and non-mast flowering events in Fraxinus excelsior
9.30 – 9.45	Burczyk, Jaroslaw Depicting seed and pollen gene flow of Dicorynia guianensis, an endemic neotropical forest tree species, on the basis of naturally established seedling
9.45 – 10.00	Burgarella, Concetta Cluster based analysis of nuclear introgression between Quercus suber and Q. ilex
10.00 – 10.30	COFFEE BREAK

Session III (cont.)

10.30 – 10.45	Grivet, Delphine Spatial genetic pattern of dispersed seedlings in the California valley oak
10.45 – 11.00	Isabel, Nathalie Gene flow between native and exotic tree species
11.00 – 11.15	Jones, Frank Measuring and modeling seed dispersal in forest trees: combining genetic and maximum likelihood approaches
11.15 – 11.30	Klein, Etienne A bayesian approach to jointly estimate the pollen dispersal function and the heterogeneity of male fecundity : Application to Sorbus torminalis
11.30 – 11.45	Oyama, Ken Hybridization process in Mexican oaks
11.45– 12.00	Robledo-Arnuncio, Juan J. A Novel Indirect Method of Estimating the Pollen Dispersal Curve that is Independent of Effective Density
12.00 - 12.15	Salvini, Daniela Gene flow in a Mediterranean oak complex: the effects of space and time
12.15 – 12.30	Smouse, Peter Measuring Pollen Structure of Enterolobium cyclocarpum for a Tropical Dry Forest Landscape in Costa Rica
12.30 – 14.00	LUNCH

Session III (Cont.)

14.00 – 14.15	Sork, Victoria Spatially explicit analysis of rangewide genetic structure in a California endemic tree species using chloroplast and nuclear microsatellites
14.15 – 14.30	Valbuena-Carabaña, María Fine-scale spatial genetic structure in mixed oak stands with different levels of hybridisation

14.30 – 14.45	Waldmann, Patrik A Bayesianspatial method for inference of the geographical distribution of allele frequencies

Session IV
Quantitative genetics, QTL studies and adaption

Chairs: R. Alía and J. Bousquet

14.45 – 15.00	Almeida, Maria Helena Quercus suber geographic variation: preliminary results of the Iberian Península provenance trials
15.00 – 15.15	Austerlitz, Frederic Evolution of genetic diversity and differentiation in metapopulations for genes involved in regulation networks
15.15 – 15.30	Diaz Vazquez, Raquel Genetic variation of spring cold hardiness in Spanish wild populations of Castanea sativa
15.30 – 15.45	Holliday, Jason Temporal monitoring of autumn gene expression within and among populations of Sitka spruce reveals candidate genes for adaptation to local climate
15.45 – 16.00	Jump, Alistair Adaptive response to climate change: warming-linked spatial and temporal trends in gene frequency in Fagus sylvatica
16.00 – 16.30	COFFEE BREAK

Session IV (cont.)

16.30 – 16.45	Knürr, Timo Migration, selection and clines in adaptive variation in Pinus sylvestris
16.45 – 17.00	Rohde, Antje An integrated approach to bud set in poplar: phenotypes, candidate genes, and QTLs
17.00 – 17.15	Taylor, Gail` Why Are Some Leaves Big? Linking Transcript, Qtl And Ld Approaches To Understand The Genetic Control OF LEAF SIZE AND SHAPE IN POPULUS

17.15 – 17.30	Villani, Fiorella An example of an integrated approach to assess the genetic and adaptive variation in populations of Castanea sativa Mill
18.00	Poster Session II

Wednesday, October 4th
Workshops – A
Association mapping in forest trees

Chairs: D.B. Neale and Santiago C. González-Martínez

8.30 – 8.40	Introductory talk (David B Neale, University of California, Davis)
8.40 – 9.00	González- Martínez, Santiago Association genetics for wood property traits in loblolly pine
9.00 – 9.20	Neale, David B. ADEPT1 – Association genetics for disease and drought traits in loblolly pine
9.20 – 9.40	Lee, Jennifer ADEPT2 – Re-sequencing and SNP discovery in loblolly pine
9.40 – 10.00	Porth, Ilga Report on the progress in the pine family comparative genomics project
10.00 – 10.30	COFFEE BREAK
10.30 – 10.50	Garnier-Géré, Pauline Association genetics projects in European forest tree species
10.50 – 11.10	González-Martínez, Santiago Association genetics for wood-, drought- and photoperiod-related candidate genes in maritime pine (Pinus pinaster Aiton): preliminary results .
11.10 – 11.30	Bousquet, Jean A SNP-based genetic map of expressed regulatory genes in the conifer white spruce
11.30 – 11.50	Pande, Barnaly Association genetics for adaptive traits in Douglas-fir

11.50 – 12.10	Taylor, Gail Sylleptic branching as an adaptive trait in Populus: QTL, candidate genes and association genetics
12.10 – 12.30	Zaina, Giusi Using an association (linkage disequilibrium) mapping approach for phenological traits in Populus nigra
12.30 – 13.00	Wrap-up of the workshop

Workshops - B
Simulation models of tree population genetics

Chairs: F. Austerlitz and J.J. Robledo Arnuncio

8.30 – 8.40	Introductory talk (Juan J Robledo-Arnuncio, Université Montpellier II)
8.40 – 9.10	Kuparinen, Anna AMELIE - A simulation model linking spatio-temporal population-genotype dynamics of trees
9.10 – 9.40	Oddou, Sylvie Ecological factors shaping the genetic quality of seeds and seedlings in forest trees: a simulation study coupled with sensitivity analyses
9.40 – 10.10	Navascués Melero, Miguel de Coalescent simulations for the evaluation of statistical tools for demographic inference and the effect of homoplasy in chloroplast microsatellites
10.10 – 10.30	COFFEE BREAK
10.30 – 11.00	Pyhäjärvi, Tanja Demographic history of Pinus sylvestris inferred from nucleotide diversity data
11.00 – 11.30	Hardy, Olivier Spatial distribution of tree species in tropical forests under a neutral hypothesis
11.30 – 12.00	Strand, Allan Indirect estimation of long-distance dispersal characteristics using spatially explicit individual-based simulation of metapopulation dynamics in a linearly dispersed plant

12.00 – 12.30	Meirmans, Patrick A metapopulation model for the introgression from genetically modified plants into their wild relatives
12.30 – 13.00	Wrap-up of the workshop
13.00 - 14.00	LUNCH
14.00	Field trip I Valsaín (conifer forest) Field trip II Hayedo de Montejo (oak forest)
18.00	Tourist visit around Alcalá de Henares

COST E-28 GENOSILVA: European Forest Genomics Network
Final Meeting

Thursday, October 5th

9.00– 9.15	Conference Opening

Session I
Functional Genomics of Tree Health

Chairs: G. Taylor

9.15 - 10.00	Markku Aalto Using candidate genes for studying season-related responses in birch (Betula pendula Roth)
10.00 – 10.30 COFFEE BREAK	
10.30 – 10.50	Emiliani, Giovanni Gene expression profiling of Populus alba in response to ultraviolet–B radiation
10.50 – 11.10	Street, Nathaniel A genetical-genomics approach to understanding abiotic stress response in Populus
11.10 – 11.30	Graham, Laura Leaf development and senescence in levated CO_2 : linking transcriptomics and QTL analysis
11.30 – 11.50	Sperisen, Christoph Involvement of a copper chaperone (CCH) in the response of poplar to cadmium
11.50 – 12.10	Faivre Rampant, Patricia Association mapping of resistance to rust disease in poplar
12.10 – 12.30	DISCUSSION
12.30 -14.00	LUNCH

Session II
Functional Genomics of Tree Maturation and Reproduction

Chairs: M. Baucher

14.00 - 14.20	Fladung, Matthias Genetic approaches to alter tree growth
14.20 - 14.40	Dillen, Sophie Leaf determinants of productivity in poplar depend on environmental conditions and genetic background
14.40 – 15.00	Heinze, Berthold A ring test for identity and distinctness in forest tree seedlots
15.00 – 15.20	Allona, Isabel Winter dormancy, cold acclimation and circadian clock in chestnut
15.20 – 15.50	COFFEE BREAK
15.50 – 16.10	Berdasco, Maria Epigenetics, the role in plant differentiation and development
16.10 – 16.30	Wilheim, Eva Differences and common features of developing somatic and zygotic embryos of oak (Quercus robur L.)
16.30 – 16.50	de Miguel, Celia Molecular approaches to understand regulation of embryo development in maritime pine (Pinus pinaster)
16.50 - 17.10	Díaz-Sala, Carmen Adventitious rooting genes in distantly-related forest species Poster Session
21.00	Conference Dinner

Friday, October 6th

Session III
Functional Genomics of Wood Formation

Chairs: P. Label

8.30 – 8.50	Sundberg, Björn Hormones and wood formation
8.50 – 9.10	Racchi, Milvia Analysis of gene expression in cambial zone of poplar (P. alba) in response to water deficit
9.10 – 9.30	Kumar, Manoj High-resolution transcript profiling across tension wood developmental zones
9.30- 9.50	Sanchez, Leopoldo In situ genetic variability estimation of wood, phenology and morphological traits in a natural stand of Quercus
9.50 – 10.30	COFFEE BREAK
10.30 – 10.50	Paiva, Jorge Molecular and phenotypic characterisation of wood forming tissues along a cambial-age gradient in maritime pine
10.50 – 11.10	Jones, Brian Unravelling the vascular cambium in Poplar and Arabidopsis
11.10 – 11.30	Label, Philippe First insight into microgenomics of cambial activity per cell type in poplar
11.30 - 12.15	Closing Lecture Birot, Yves (Chairman of the Scientific Council of the Forest-based Technology Platform) The Forest-Based Technology Platform in the context of European Forest-related Research
12.15 – 12.30	Conference Closing
12.30-14.00	LUNCH
14.00	Management Committee Meeting

IUFRO

PLENARY CONFERENCE

Recent Developments In Conservation Genetics.

Philip Hedrick

Genetic variation may be divided into neutral, detrimental, and adaptive components and I will discuss examples for detrimental variants from Florida panthers and Mexican wolves. Understanding both population structure and effective population size are fundamental to conservation genetics. I will illustrate various aspects of these concepts by application of neutral markers and simple theoretical considerations. (1) Measuring population structure using highly variable markers, such as microsatellites, may give values that are smaller than for markers with less variation. I will discuss a new standardized measure that is appropriate for different types of genetic markers and different species. (2) Often the past population size of endangered species is not known or difficult to estimate. Using museum samples from around 1890, the bottleneck size of Florida panthers was estimated as around two individuals for two consecutive generations.

NOTES:

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(*Post*-) genomic studies of species barriers: from annual plants to forest trees

Christian Lexer[1]

Recent developments in forest tree genomics greatly enhance our ability to understand the factors that create or maintain species barriers, or barriers to gene flow between divergent populations. Genome-wide marker surveys increasingly allow forest geneticists to study how levels of gene flow between populations or species may vary among different loci in the genome. This allows geneticists to disentangle factors affecting the entire genome from the locus-specific footprint of selection. This 'population genomic' approach promises new insights into the genetic basis of adaptation and species differentiation in perennial plants. When applied to species of economic relevance, it may also have important 'spin-offs' in tree improvement and breeding. Recent experimental work on this topic will be reviewed. This will include recent work on the origin of ecological divergence in hybridizing annual taxa (Helianthus spp.; sunflowers), which may inform related work in long-lived trees. It will also include recent work on the potential for non-neutral introgression in European members of the 'model tree' Populus (poplars and aspens), and a trans-disciplinary study on palms (Howea spp.) providing the perhaps most convincing evidence to date for sympatric speciation in plants. Previously under-explored issues will be highlighted, such as the potential of spatial genetic structure (SGS) analysis in studies on the evolution of reproductive barriers.

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KEYWORDS:
hybridization, gene flow, selection, speciation, Populus

Evoltree: Evolution Of Trees As Drivers Of Terrestrial Biodiversity

Antoine Kremer

Evoltree is a Network of Excellence (NoE) granted last year by the European Commission (Priority 6: Global Change and Ecosystems). NoEs are instruments to overcome the fragmentation of the European research landscape with the objective to strengthen European excellence in a given area. Their purpose is to reach a durable restructuring/shaping and integration of efforts and institutions where this is necessary. To reach their goals, NoEs include not only research activities, but also integration and dissemination activities. NoEs were created for the first time within the 6th framework programme (2002-2006). In the area of population genetics of trees, **Evoltree** should therefore be seen is as a reinforcement and enhancement of European support within earlier framework programmes. **Evoltree** is a consortium comprising 25 partners from 15 different European countries, and has been launched last April.
Evoltree will implement genomic and molecular approaches for understanding, monitoring and predicting genetic diversity, ecosystems structures, dynamics and processes in terrestrial ecosystems. Research in **Evoltree** has essentially three major components: trees, associated species (insects, fungi..) and their interactions.

The objectives of the research activities are:

- Identify genes of adaptive significance in regards to global change in three model species of trees (Pinus, Populus and Quercus), phytophagous insects (Limantria) and mycorrhizal fungi (Laccaria and Glomus)
- Assess the level and distribution of nucleotide diversity in genes of adaptive significance in trees, insects and mycorrhizal fungi.
- Assess the impact of trees on the composition of communities by studying interactions between trees and their associated species.
- Investigate the evolutionary rate of trees by reconstructing their past history and predicting their future response to global change.

The network will capitalise on the substantial expertise and availability of genomic resources accumulated in different European countries during previous collaborative projects. It will integrate interdisciplinary research to decipher the structure, expression and polymorphism of genes of adaptive significance. The extant distribution of diversity will be integrated into an evolutionary perspective.
The genomic activities will be conducted within a ‘virtual lab’ where high throughput techniques will be integrated and then applied to a wider range of tree and associated species, starting with the model species. **Evoltree** will install and enhance the necessary experimental infrastructures (repository centers, Intensive study sites..), information systems and bioinformatic resources for common use by the partners. Large data sets will be compiled and made accessible by developing data mining procedures for the analysis of geographic and temporal distribution of genetic diversity. **Evoltree** will spread its knowledge and expertise for the purpose of education, ecosystem monitoring, and the development of biodiversity conservation strategies. The network will develop training capacities and facilitate mobility opportunities throughout Europe.

NOTES:

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SESSION I

GENE DIVERSITY, PHYLOGEOGRAPHY AND POPULATION GENOMICS

I-SI,1

Clinal patterns of nucleotide diversity and differentiation at candidate genes for drought stress tolerance and bud phenology in a maritime pine metapopulation

Carmen Collada[1,2], M. Ángeles Guevara[2,3], Álvaro Soto[2,4], Santiago González-Martínez[3], Luis Díaz[3] , Barbara Vornam[5], Oliver Gailing[5], Reiner Finkeldey[5], Valérie Léger[6], Christophe Plomion[6] and *M. Teresa Cervera*[2,3],

Forest trees are long-lived organisms, with a life continuously challenged by changing environmental conditions, such as drastic climate changes. Survival of tree species is associated to their ability to adapt to new environmental conditions, which depends on the genetic variability of genes involved in the control of adaptive traits, and on their plasticity. DIGENFOR is a multidisciplinary project that aims to identify mutations of adaptive significance based on nucleotide diversity pattern analysis in non-model forest tree species such as maritime pine (Pinus pinaster Aiton). Natural populations have been sampled along a rainfall gradient in central Spain in order to assess intra- and inter-population nucleotide diversity at fragments of 22 candidate genes involved in fitness-related traits such as bud phenology and drought stress response. Preliminary results reveal similar nucleotide diversity estimates than those obtained in previous functional and expressional candidate studies of pines and other conifer species. Several genes have non-synonymous substitutions at a relatively high frequency (> 0.3) which may be indicative of natural selection. Candidate gene studies lead to hypothesize different molecular evolution scenarios. SNPs of adaptive value will be validated from their associations with the phenotypic variation of adaptive traits.

NOTES:

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KEYWORDS:
adaptation; nucleotide diversity; Pinus pinaster; drought stress; phenology

Molecular genetic differentiation at candidate genes for drought stress tolerance and microsatellite markers in Pinus pinaster natural populations

***Emmanuelle Eveno*[1], Carmen Collada[2,3], M. Angeles Guevara[3,4], Álvaro Soto[3,5],**
Santiago C. González-Martínez[4], M. Teresa Cervera[3,4], Christophe Plomion[1],
Pauline H. Garnier-Géré[1]

Genetic differentiation has long been studied to better understand the process of divergent selection, along with that of other interacting evolutionary factors in natural populations. Several outliers-based tests using genetic differentiation have been developed, and are currently but mostly applied in model species, using both genome-wide and population samplings (humans, drosophila, arabidopsis…). A greater focus on explicit sampling of distinct local populations has been recently advocated as a promising approach for detecting positive selection events in non-model species. Here we present results from an outlier's-based approach using both gene and nucleotide levels differentiation estimates in at 12 candidate genes for drought stress tolerance, and molecular differentiation at eight microsatellites considered as reference neutral markers. The material studied includes ten natural populations of maritime pine, which also follow a rainfall gradient. Genetic differentiation was estimated for both candidate genes and microsatellites by testing 2 types of clustering. The first one based on microsatellites data used as a neutral reference, will allow to discriminate between selective and demographic causes of differentiation. The second one matching rainfall and temperature gradients, intends to help interpret further eventual outlier patterns that could be due to differential adaptation related to climatic conditions. Genetic differentiation was estimated for both candidate genes and microsatellites by testing 2 types of clustering. The first one based on microsatellites data allows to discriminate between selective and demographic causes of differentiation. The second one matching rainfall and temperature gradients, intends to help interpret further eventual outlier patterns that could be due to differential adaptation related to climatic conditions. Significant deviations from neutrality were observed for 3 genes involved in cell wall formation and general stress response. Within these genes, 16 outlier SNPs have been detected that show FST values ranging from 40% to 80%, which are interpreted as a possible consequence of positive selection events. In contrast, another gene belonging to the dehydrin family exhibits much lower differentiation than neutral markers, which could indicate balancing selection. Among these genes, several non-synonymous SNPs can be proposed as good candidates for future association study at the between population level.

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KEYWORDS:

nucleotide differentiation, drought stress tolerance, Pinus pinaster, candidate genes, rainfall gradient

The demographic history of Shorea parvifolia (Dipterocarpaceae) inferred from nucleotide polymorphism at multiple nuclear genes

H. Ishiyama[1], I. Khatab[1] R. Finkeldey[2], N. Inomata[1], Y. Tsumura[3], T. Yamazaki[4], A. E. Szmidt[1]

The fossil data suggest that at the Last Glacial Maximum (approximately 20,000 years ago), savanna vegetation was widespread in Southeast Asia, and rainforest refugia were present only on the northern and eastern Borneo, northern and western Sumatra and the Mentawai islands. At present, the rainforests distribute in Peninsular Malaysia and whole Sumatra and Borneo islands. Thus, tropical tree species in Southeast Asia might have experienced changes in the distribution and population size. To investigate the demographic history of tropical tree species and its effects on their genetic diversity, we analyzed the levels and patterns of nucleotide polymorphism for the SBE2 and PgiC nuclear gene regions in Shorea parvifolia. Shorea parvifolia is a dominant tree species in the lowland tropical rainforest in Southeast Asia. To cover most of the natural range of S. parvifolia, samples were collected from two locations in Peninsular Malaysia, two locations on Sumatra island and three locations on Borneo island. We found considerable population differentiation (pairwise $F_{st} = 0.2$ to 0.4) between populations from western region of Southeast Asia (Peninsular Malaysia and Sumatra) and populations from Borneo island, but weaker differentiation within the regions. These results possibly reflect the separated distribution of rainforest on Sumatra and Borneo during the Pleistocene (0.01 to 1.6 Mya). Furthermore, the results of mismatch distribution analysis suggested that most of the investigated populations experienced past expansion and that the expansion event in Borneo populations occurred earlier than that in populations from western regions.

NOTES:

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KEYWORDS:
tropical tree, population genetics, demographic history, glacial periods, nucleotide polymorphism

A SNP genome scan for the search of gene polymorphisms involved in adaptive population differentiation in white spruce

Marie-Claire Namroud[1], **Jean Beaulieu[1,2], Jérôme Laroche[3], and Jean Bousquet[1]**

The main objective of this study was to assess, for the first time, the effectiveness of a wide genome scan approach involving SNPs of regulatory genes in detecting local adaptation in a coniferous species. For this, we sampled between 20 and 34 individuals from six natural populations of white spruce (Picea glauca) previously shown to be differentiated for quantitative characters in progeny tests (Q_{ST}). We screened 534 single nucleotide polymorphism (SNP) markers representative of 425 expressed genes which are distributed across the 12 linkage groups on the genome of white spruce. Expected heterozygosity and within-population fixation index F_{IS} were estimated. Identification of candidate loci under selection was conducted by estimating the among-population differentiation (F_{ST}). F_{ST} was also simulated with a Bayesian regression model based on the likelihood of allele frequencies. This model has the advantage of modelling separately the forces acting at the genome and population levels. Candidate loci under selection were identified by outlier analysis. Most SNPs (83%) were selectively neutral, in agreement with the neutral theory model and as expected for a wind pollinated species occupying a wide natural range such as white spruce. Outlier analysis made it possible to identify 61 candidate SNPs under putative selection. The bulk of these SNPs (70%) represented gene loci putatively involved in growth, growth rhythm, or phenology. Such trend is consistent with among-population patterns of quantitative variation, which showed higher values of Q_{ST} for traits related to phenology. About 15% of SNPs were locally adapted to a population located in a high altitude region with above average snow falling, which is likely indicative of adaptation to colder climate conditions.

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KEYWORDS:
genome scan, SNP, white spruce, outlier analysis, natural selection.

Genetic variation and differentiation of the green oak leaf roller (*Tortrix viridana* L.) and its host (*Quercus robur* L.)

Hilke Schroeder, Florian Scholz, Bernd Degen

In Western Europe pedunculate oak (Quercus robur L.) is the forest tree with the highest number of phytophagous insect species. One of these, the green oak leaf roller, Tortrix viridana L. (Lepidoptera, Tortricidae) is an oligophagous herbivorous moth with a host range limited to the genus Quercus. Its distribution area corresponds with that of its host species. During outbreaks, T. viridana often leads to defoliation of oaks in spring. The abundance of T. viridana is subject to the population size fluctuations typical for herbivorous insects, where periods of small population sizes (latent periods) alternate with periods of high population sizes (outbreak).

Former experimental studies were focused on population dynamics of the moth, but so far only little attention has been paid to the genetics of the species. The partitioning of genetic variation within and among populations of Tortrix viridana is mainly controlled by migration, and thus of great interest to forest management and protection, since information on migration is important to predict future pest outbreaks and expansion events.

Here we present first results of genetic differentiation among populations of T. viridana and its host using AFLP markers. We focused on the differences and similarities between insect and host on a geographic scale up to 200 km. We found slightly higher genetic differentiation for the oak populations compared to the green oak leaf roller. First results on genetic differentiation of the green oak leaf roller over the whole distribution area in Europe showed stronger genetic structure that can be explained by the isolation by distance model.

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KEYWORDS:
Tortrix viridana; Quercus robur; AFLP; population genetics

Patterns of nucleotide polymorphism in the gMdh nuclear gene region of Eurasian Larix (Pinaceae) species

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Nucleotide variation of a nuclear gene coding for glyoxysomal malate dehydrogenase (gMdh) was investigated in six Eurasian Larix species and their two varieties. The sequences of haplotypes for 84 individuals were obtained for the 1285 bp gMdh gene region. Based on the constructed networks and distribution of polymorphic sites, two major diverged haplotype groups (designated as I and II) were detected. Haplotypes from the group I differed by only one or two nucleotides. On the other hand, haplotypes from the group II were more diverged but had common nucleotides at several sites. The distribution of the two groups showed clear geographic pattern. Populations from northeastern and central Siberia harbored only haplotypes belonging to group I. On the other hand, haplotypes from both groups were present in populations from southern Siberia, northern Kazakhstan, and western parts of the Larix distribution. High nucleotide diversity (π) and population differentiation (F_{st}) were detected in populations from some regions. A very high differentiation was detected between three geographically adjacent populations of Larix olgensis. It is possible that it is result of past admixture from diverged ancestors or the presence of other species in the region occupied by L. olgensis. This hypothesis is supported by evidence for the presence of multiple refugia in southern Eurasia.

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KEYWORDS:
Larix, phylogeography, nucleotide polymorphism

Genetic diversity and linkage disequilibrium in drought tolerance candidate genes in Aleppo pine (*Pinus halepensis Mill*)

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Single nucleotide polymorphisms are emerging as an important tool for the assessment of genetic diversity and, if detected in candidate genes, may allow gathering information about adaptive variation. Aleppo pine (Pinus halepensis) is an outcrossing highly heterozygous species, with very large effective population sizes. In the present study we report on SNP discovery, level of nucleotide polymorphism, haplotype structure and linkage disequilibrium for nine candidate genes related to drought stress tolerance in natural populations of Aleppo pine. Six natural populations were sampled along a geographical and ecological gradient (Greece, Israel, Algeria, Morocco, Spain and Italy). The nine candidate genes were amplified in at least eight individuals per population and sequenced from both ends. The overall nucleotide variation was limited and lower than that observed in most tree species so far studied. Tajima's D values across loci tend to be negative, thus indicating the presence of an excess of rare variants compared to the expectation according to a standard neutral model, which might be related to range expansion. Tests for genetic differentiation among populations revealed a significantly high F_{st} value for some candidate genes, much higher than those estimated in Aleppo pine for neutral markers (isozymes and chloroplast microsatellites). The extent of linkage disequilibrium within some candidate genes, estimated by r^2, showed the tendency of a rapid decline within few hundreds of base pairs, as already observed in other conifer species, which are typically outcrossers (e.g., Picea abies). Possible implications of these results in conservation genetics and association mapping studies are discussed.

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KEYWORDS:

nucleotide diversity, linkage disequilibrium, population structure, Mediterranean Basin, Aleppo pine

Adaptive variation for cold tolerance and nucleotide diversity for cold candidate genes in Scots pine (*Pinus sylvestris*)

Witold Wachowiak[1], Katri Kärkkäinen[2], Tapani Repo[3], Outi Savolainen[1]

The steep clines of many phenological traits in conifers are likely results of natural selection. The nearly undomesticated, random mating populations of Scots pine with low geographic structure and wide Eurasian distribution range are excellent experimental systems for genetic analyses of adaptive phenotypic variation. We applied a candidate gene approach to study the genetic basis of the adaptive variation for cold tolerance. Phenotypic measurements of frost resistance were conducted in a common garden experiment on seedlings from seven populations including three Finnish, Swedish, Scottish, Polish and Turkish populations. The steepest part of the cline to cold tolerance was between latitude 50° N and 67° N ranged from – 60C to –210C between Central European and North Finnish populations. The level of nucleotide diversity was analyzed in fourteen genes including several members of dehydrin gene family. DNA samples of haploid megagametophyte tissues from 40 unrelated individuals derived from eight geographically distant populations were used in SNPs discovery panel. Different levels of nucleotide polymorphism and very rapid decay of linkage disequilibrium (within ~500bp) was observed among the genes. The selected SNPs distinguishing both the most common haplotypes and the rare haplotypes marked by nonsynonymous substitutions will be screened in full range samples phenotypically characterized as a part of association studies that aim at identifying genes underlying cold tolerance in Scots pine.

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KEYWORDS:
adaptation, latitudinal cline, nucleotide polymorphism, linkage disequilibrium, candidate genes

SESSION II

CONSERVATION GENETICS

I-SII,1

Genetic processes during natural regeneration within the mixed oak stands

Igor J. Chybicki **[1,3], Jaroslaw Burczyk[1], Artur Dzialuk[1], Magdalena Trojankiewicz[1], Adam Boraty_ski[2], Andrzej Lewandowski[2]**

We investigated patterns of gene flow taking place during natural regeneration within the two mixed oak stands of Quercus robur and Q. petraea located in northern and southern part of Poland, using a novel maximum likelihood method, the Seedling Neighbourhood Model. The analyses were based on 421 and 290 adult trees and 295 seedlings in both stands, which were genotyped with a set of 6 nSSR markers. The study showed that an effective gene flow through acorns is very restricted in oaks. Most seedlings were mothered by nearby trees and the average distance between seedlings and their mothers was 9.4 and 4.4 meters within the respective stands. The effective seed immigration from outside of the plot (the distances larger than 50 m) reached 4.5% and 7.4% in the two stands. On the other hand, effective gene flow through pollen was much more extensive, as might be expected for wind-pollinated tree species. The rate of pollen immigration from males located further than 50 m from females was estimated to be about 60% in both stands; however, a low but significant negative correlation between the probability of mating and the distance between mates was detected. The mean effective pollen dispersal distance within the respective stands was found to be 22.6 and 17.9 meters. Apparently, a few seedlings resulted from self-fertilization, nevertheless a detailed parentage analysis revealed that all selfed progeny was mothered by a single pedunculate oak tree. W also detected some seedlings being the product of inter-specific matings (3.5% and 11.5%, in respective stands). However, it remains unclear whether the observed effective "hybridization" occurred between individuals of pure or introgressed species.

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KEYWORDS:
gene flow, hybridization, nSSR, maximum likelihood estimation, Quercus sp.

I-SII,2

Genetic diversity in Mediterranean forest patches: A case study.

***Laura F. Carrillo*, Rafael G. Albaladejo, Juan Pedro G. Varo, Abelardo Aparicio.**

Recently, many studies have focused in the (genetic) consequences of habitat loss and fragmentation specially in tropical and temperate forests. Comparatively, the mediterranean forest in the Mediterranean Basin received much less attention likely due to intense antropogenic management since 2000 BP or even more. Here, we present the result of a comprehensive study trying testing the effects of habitat fragmentation in four shrub species (Myrtus communis, Quercus coccifera, Pistacia lentiscus and Cistus salviifolius) with contrasting life-history traits (i.e. mating system and dispersal) in southwestern Spain. Isozyme electrophoresis were conducted to assess levels of genetic diversity and Geographic Information Systems (GIS) were used to obtain a set of predictor variables related to patch features, isolation metrics and changes in patch and landscape configuration happened since 1956. Then, we performed generalized linear methods to generate multiple predictor models and use a model selection approach based on information theory to test the effect of predictors on genetic diversity in the four species.

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KEYWORDS:
Akaike's information criteria; generalized linear methods; Geographic Information Systems (GIS); habitat fragmentation; isozymes.

Estimating heritability from molecular marker information: the accuracy of a regression approach

Silvia Teresa Rodríguez-Ramilo[1], Miguel Ángel Toro[1], Armando Caballero[2] and *Jesús Fernández*[1]

The heritability of a quantitative trait is a key tool to quantify the amount of genetic variation present in a population. Although estimates of heritability require accurate information on the relationship among individuals, pedigree data is generally lacking. Nowadays, the increasing availability of DNA markers is making possible the estimation of coancestries from neutral molecular information. In 1996, K. Ritland developed an approach to estimate heritability based on the regression of the phenotypic similarity on the estimated coancestry. We carried out simulations to evaluate the accuracy and behaviour of the narrow sense heritability estimated by this method under different population scenarios. Accuracy of the estimated coancestry was also tested to infer if it has implications on the performance of the estimated heritability. The heritability of a quantitative trait controlled by 120 additive, biallelic loci was estimated using information from 10, 20 or 100 codominant neutral molecular markers. Simulations involved census sizes of 50 or 500 individuals with equal number of males and females. Population size was constant along 10 discrete generations in two possible scenarios: (1) populations with no family structure, where contributions of parents to progeny were free and mating was at random; (2) populations with a family structure, where 5 or 50 full-sib families, depending on the population size, were obtained through equal contributions of parents to the next generation (10 offspring per family), and parents of each family were chosen at random or selected according to their phenotypic value. The mating procedure in the second scenario was at random or assortative according to the genealogical coancestry. Our results suggest that the two main factors affecting the accuracy of the heritability estimator are the absence of a familiar structure in the population, and the selection on the evaluated trait. Nevertheless, for large population sizes and a considerable number of molecular markers the heritability estimator shows a suitable behaviour. Finally, it is also concluded that the coancestry estimated from molecular information is likely to be more suitable to infer coancestry than inbreeding and its accuracy depends on the variance and dispersion of the genealogical coancestry values.

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KEYWORDS:

heritability, quantitative trait, kinship, inbreeding, molecular marker

Phylogeography of African lowland rainforest trees: preliminary results

Myriam Heuertz[1], **Guillaume Koffi Kouamé**[1], **Vincent Savolainen**[2] **and Olivier Hardy**[1]

The climatic oscillations of the Quaternary have produced repeated shifts in the distribution ranges of plant and animal species. In equatorial Africa, wet periods with extensive forest cover alternated with arid periods during which the savannah extended and the distribution of rainforest taxa became fragmented. In the Guineo-Congolian phytogeographic region in western and central Africa, three areas display particularly high levels of species richness and endemism: the Upper Guinea area, the Lower Guinea area and the Congolian Basin. They have therefore been suggested to have held rainforest refugia during dry periods. High species richness and endemism in these regions result from speciation events due to the accumulation of differentiation at the within-species level. We investigate here whether geographic patterns of species richness and endemism are paralleled by similar patterns of genetic diversity and allelic endemism at the within-species level. We present preliminary results on the geographic distribution of sequence diversity in chloroplast DNA (cpDNA) in about 20 wide-spread African lowland rainforest trees belonging to different plant families. Due to the supposed selective neutrality of cpDNA, these data essentially reflect the demographic history of the taxa. The comparison of patterns between species will give first insights on the impact of species life-history traits shaping the patterns of genetic diversity in African lowland rainforest taxa.

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KEYWORDS:
phylogeography, central Africa, lowland rainforest, cpDNA, multi-species approach

I-SII,5

Imprints of the Holocene collapse in the modern cytoplasmic DNA population structure of threatened montane conifers from northern Mexico

Juan P. Jaramillo-Correa[1], **Erika Aguirre-Planter**[2], **Jean Beaulieu**[1,3], **F. Thomas Ledig**[4], **Luis Eguiarte**[2], **Damase Khasa**[1], **and Jean Bousquet**[1]

A dramatic population decline has been observed since the end of the Last Glacial Maximum (LGM) through previous analyses of the fossil record for most of the montane conifers of northern Mexico. These montane species were abundant and had widespread ranges during and before the LGM. However, they have retreated to higher elevations until being confined to small island-like pockets of favourable habitat following the dryness process observed in this region during the Holocene. We have surveyed the range-wide variation of different species of the gPicea and Abies genera from northern Mexico by using maternally inherited mitochondrial (mtDNA) and paternally inherited chloroplast (cpDNA) DNA markers. The average mtDNA and cpDNA diversities were extremely low for all these taxa when compared to their congeneric counterparts from northern North America and southern Mexico. The distribution of mitotypes revealed clear non-overlapping areas (G_{ST} -values between 0.8 and 1), suggesting a previous subdivision of ancestral populations and/or a very rapid and geographically structured fixation of mitotypes within the collapsing stands. The cpDNA markers showed lower population differentiation (G_{ST} -values between 0.20 and 0.65) than the mtDNA markers, implying that the ancestral populations continued to exchange pollen after their initial geographic separation, though at a very limited pace. In addition, different spatial analyses ($G_{ST} > R_{ST}$; and SAMOVA) revealed a lack of a phylogeographic structure for cpDNA markers, suggesting that the modern population genetic diversity of these taxa is essentially modeled by genetic drift. The reduced levels of cpDNA gene flow noted among populations of the same species, and the absence of mutation-drift equilibrium observed for some of these stands; point to recent bottlenecks and to effective population isolation in several instances. The various gene diversity trends observed here are consistent with the hypothesis of population collapse during the Holocene warming for all the species surveyed, which should be taken into account for the establishment of conservation programs. Similar studies on other threatened montane tree species may also be useful to understand the genetic effects of the glacial periods on such species when confined to small populations.

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Genetic studies of native *Malus sylvestris* populations in Denmark: is the local gene pool endangered?

***Anders Søndergaard Larsen*[1], Conny Bruun Asmussen[2], Els Coart[3], Martin Jensen[4], Ditte Christine Olrik[1], Erik Dahl Kjær[1]**

The European crab apple (Malus sylvestris (L.) Mill.) is a small insect pollinated tree of the family Rosaceae, indigenous to the European continent exclusive of the southwestern part and northern Scandinavia. The genetic resource of Malus sylvestris is considered endangered in Europe - the main threats being identified as (1) fragmentation and reduction of its habitat; potentially associated with genetic erosion and increased autozygosity (inbreeding) through genetic drift, (2) hybridization with the domesticated apple Malus xdomestica Borkh.; potentially associated with outbreeeding depression and loss of genetic integrity.

We approached the hybridization issue by comparing 178 M. sylvestris individuals from four Danish populations to a sample of 29 old cultivars of M. xdomestica. By looking at morphological characters and variation at ten SSR loci we concluded that hybridization between the two species is less frequent than previously anticipated, although morphological character traits did indicate that some introgression has taken place since the introduction of M. xdomestica to Denmark some 800 years ago. We made controlled crossings between eight clones of M. sylvestris and five cultivars of M. xdomestica. The results indicated no genetic incompatibility between the two species, because the fertilization success of interspecific crosses was not lower than in intraspecific crosses made as reference in M. sylvestris. However, a comparative assessment of flowering phenology in two native M. sylvestris populations and nearby orchards of M. xdomestica revealed that the latter flowered slightly later in the year of study, indicating that some isolation between the species due to timing may occur; potentially reducing the level of hybridization. Spatial pollen distribution in situ was studied in one natural population of M. sylvestris based on genotyping of progenies from randomly distributed seed trees. Pollinations were mostly observed between nearby trees, indicating that isolation by distance could be an important isolating factor between M. sylvestris and M. xdomestica. This is consistent with the fact that we found the highest number of hybrids in populations that are known to have been weakly isolated by distance from M. xdomestica. Based on traditional F-statistics, we further found that fragmentation has not yet played a major role for the four native M. sylvestris populations studied, because F_{st} was estimated to be quite low and the levels of diversity were fairly high. In conclusion, our results suggest that it is still possible to locate genetically authentic populations of M. sylvestris in Denmark for conservation and propagation measures. Implications for conservation and genetic resource management are discussed

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KEYWORDS:

Hybridization, crossability, gene flow, microsatellite, phenology.

Extinction, trans–oceanic dispersal, and gene flow in a tropical wetlandforest species

Alejandro Nettel[1], **Richard S. Dodd**[2], **Zara Afzal–Rafii**[3]

Understanding population level processes that shaped the genetic diversity and differentiation of tropical species is one of the main goals of evolutionary biology. The widespread distribution of the black mangrove, Avicennia germinans L. (L.) (Avicenniaceae), a key component of mangrove forests across the Americas and West Africa, makes it an ideal system to study trans–Atlantic dispersal and the effect of past climatic changes in a tropical species. Divergence time estimations based on ITS sequences and chloroplast DNA geographic distribution show trans–oceanic dispersal during the Quaternary. Low diversification and genetic diversity estimates for populations at the northern range limits and in some mid-Atlantic regions suggest recolonization after major extinctions due to climate and sea level change. A detailed gene flow study in the genetically diverse East Pacific using nuclear microsatellite loci shows low migration, suggesting that despite the long distance seed dispersal potential of the species, establishment may be prevented by locally native populations. We discuss our results and their repercussions in light of our understanding of mangrove ecosystem evolution, as well as their implications for the conservation of this fragile ecosystem.

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Heritabilities out of the beaten test site: looking at the perspectives of in situ inferences for forest tree species with a preliminary study in *Quercus* species.

L. Sanchez **[1]; S. Gerber [2]; J. C. Vilardi [1,4]; A. Ducousso [2]; J. L. Puech [3]; C. Rathgeber [5]; P. Rozenberg [1]**

With non domesticated species, like most forest trees, the assessment of heritability for traits of adaptive relevance represents one of the first steps granting future conservation and breeding programs. This essential step, however, is lengthy to conduct on long-lived species, implying mostly ex situ experiments and little environmental resemblance to the original populations. A major obstacle for in situ genetic studies has been the difficulty in grasping the extent of family interrelations in natural populations. Recent developments in molecular biology, however, are making genetic markers readily available and more efficient for measurement of relatedness. Theoretical improvements have also been proposed to infer the heritability and the evolution of genetic variance from the joint analysis of natural relatedness and the in situ quantitative trait variation. Here we propose one of the first evaluations with forest tree species of this in situ approach. The studied population comprised 280 trees of two interbreeding species: Quercus petraea and Quercus robur. This study considered as many as 60 different traits: foliar morphology, growth, phenology, wood properties, wood defects and wood biochemical compounds, some of which with no published records of heritability estimations. A total of 17 highly polymorphic microsatellite markers were studied in the sample. The genetic markers revealed low levels of relatedness and variance of relatedness, which are in principle unfavourable conditions for the detection of heritability. We proceeded by ranking the estimates among traits, and carried out randomization test to setup confidence intervals. We found that those traits known to be highly heritable in the species or at least in related taxons (i.e. phenology, wood physical and biochemical properties) appeared at the top of the trait ranking for the two species, with highly significant associations between the genetic distance revealed by the genetic markers and the phenotypic resemblance. On the opposite, other traits known to have moderate heritabilities, like growth, showed positive but no significant associations. Moreover, the two species exhibited different heritability patterns for leaf morphology traits, known to discriminate Quercus species. Although this in situ methodology cannot substitute its more precise ex situ counterpart, it could become an excellent exploratory tool of the natural occurring variation, and the detection of valuable populations for conservation purposes. We discuss the role of some useful add-ons that might further improve this in situ approach, like spatial statistics and simulation studies.

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KEYWORDS:
Heritability, natural population, microsatellites, relatedness, genetic variation

I-SII,9

Molecular diversity and population structure in native ash *(Fraxinus excelsior)* populations in the United Kingdom.

***Bruce G. Sutherland*[1], Samantha Nier[1], Angjelina Belaj[1], Jason Hubert[2], Karen Russell[1].**

The consequences of planting exotic germplasm near native tree populations are largely unknown. Gene flow between the two may alter the genetic structure of local provenances, possibly to their detriment, especially in a wind-pollinated species such as ash (Fraxinus excelsior). UK government policies encourage the planting of new native woodlands using appropriate local seed provenances rather that distant sources as local material is thought likely to be more genetically similar and better adapted. As yet there is little data describing provenance genetic structure of a major tree species in detail. The UK is divided into 24 seed zones, based on geographic, ecological and climatic data. We have investigated genetic structure of native UK ash provenances, in a set of 830 individuals taken from 42 native provenances across 21 UK seed zones. They were screened with six nuclear microsatellite markers and four chloroplast markers used previously in ash. Nuclear markers found a high overall level of genetic diversity across the whole of the UK, except for a few allele-poor provenances in the far north and west of Scotland. But very little evidence was found of significant population subdivision between UK ash provenances, which is composed of a single diverse and homogenous population. Chloroplast markers identified seven distinct haplotypes, one of which was predominant and was found in all provenances. The remaining six haplotypes were much rarer and were found only in Scotland and the far north of England, and may be evidence either of ancient refugia or recolonisation events. Our molecular data suggests possible revisions of the UK seed zone map are required at least for ash. Information for adaptive traits is being gathered using seed from the same 42 provenances. Ash seedling provenance trials at four nurseries have been established and range of adaptive traits (germination, leaf flushing, tree height) being recorded. Marked differences in germination between provenances were found to be consistent across nurseries. However, no obvious correlation with seed zones was seen. Results from this work should help inform future policies regarding the selection and use of ash for native woodlands.

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KEYWORDS:
Fraxinus excelsior, seed provenance, genetic diversity, population structure.

SESSION III

GENE FLOW, SPATIAL STRUCTURE AND HYBRIDIZATION

Pollen flow and siring success in mast and non-mast flowering events in *Fraxinus excelsior*.

Sarah Rendell[1, 2] Janet Stewart[1], *David Boshier*[1]

Common ash (Fraxinus excelsior) is an important component of mixed broadleaved woodlands in Europe, and increasingly planted and managed for conservation and ecological restoration, as well as production, objectives. Ash is wind-pollinated with a complex sexual system. Flowers may be male, hermaphrodite or female in function, with a range of intermediate forms having full function in one gender but only rudimentary function in the other. Flowers types are combined in various proportions in inflorescences, producing trees with a range of gender expression.

We carried out detailed studies of paternity and pollen flow in a mapped 12 ha mixed deciduous woodland in southern England during mast and non-mast years to:

- estimate levels of outcrossing/selfing in natural populations of F. excelsior;
- calculate levels of pollen flow in ash woodlands;
- compare mating success of hermaphrodite and male trees;
- identify factors influencing siring success, such as size or age;
- compare results between years (mast and non-mast).

Using microsatellite markers we detected very high levels of outcrossing in both mast and non-mast years. Gender was the main factor determining siring success, with most seed produced by pollen from males, although pistillate trees ('females' and hermaphrodites) sired some seed. Pistillate trees sired proportionately more seed in the non-mast year when fewer potential mates were available. Distance from the mother tree and size (dbh) of the father also had significant, but small, effects on fathering success, with closer and larger males fathering more seeds than distant and smaller ones. External pollen flow into the stand was higher in non-mast years than in mast years, with seed from edge trees more often being sired from outside the stand compared to seed from trees in the centre.

Data from this and other studies show that wind dispersal of pollen and subsequent siring of seed in F. excelsior is highly dependent on the density of the stand of trees, the proportion of trees flowering, the degree of synchrony between stigma receptivity and pollen release between stands, and on the local environment and landscape. We draw recommendations with respect to seed collection practice, the design and management of seed orchards and seed stands.

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KEYWORDS.

paternity, seed collection, ash, landscape, microsatellites

Depicting seed and pollen gene flow of *Dicorynia guianensis*, an endemic neotropical forest tree species, on the basis of naturally established seedlings.

Jaroslaw Burczyk[1]**, Celine Latouche-Halle**[2]**, Henri Caron**[2]**, Antoine Kremer**[2]

Distribution of genetic variation within and among plant populations depends largely on the extent of gene flow through pollen and seeds. Understanding of the mechanisms underlying genetic diversity of offspring populations is particularly important for endemic and naturally regenerating species. We investigated the 'realized' gene flow observed at the stage of naturally regenerated seedlings of Dicorynia guianensis Amshoff (Fabaceae), an endemic canopy tree of the Guianan tropical rain forest. Earlier studies on this species indicated extensive pollen dispersal, but the pronounced spatial genetic structure suggested relatively restricted seed dispersal. Employing recently developed seedling neighborhood model we were able to describe immigration levels and patterns of local seed and pollen dispersal, based on genotypes and locations of seedlings and adult trees. The analyses were further facilitated by combining information from bi-parentally (nuclear microsatellites) and maternally (cpDNA haplotypes) inherited markers.

On average, only 0.022 (±0.021) of seedlings resulted from seed immigration from outside of a local population (i.e., seed dispersal over distances larger than 350 meters), while 0.547 (±0.059) of matings were due to long distance pollen dispersal. Although no determinants of male reproductive success were detected, the female reproductive success was positively correlated with the size of mother trees. The probability of maternity decreased with distance between seedlings and potential mothers, indicating localized seed dispersal. We also found that the level of self-fertilization among successfully established seedlings was low and not significantly different from zero. The two mechanisms, extensive pollen mediated gene flow and localized seed dispersal, allow for maintaining relatively high levels of genetic diversity within populations and the genetic cohesion of the species, but also promote the efficient adaptation of populations to local environments.

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KEYWORDS:
gene flow; pollen and seed dispersal; natural regeneration; seedling neighborhood model;

Cluster based analysis of nuclear introgression between *Quercus suber* and *Q. ilex*

Concetta Burgarella[1]**, Zaida Lorenzo**[1]**, Álvaro Soto de Viana**[1]**, Luís Gil**[1]

Understanding hybridization dynamics is of great concern in evolutionary studies as well as in conservation biology and resource management. Patterns of introgression have been studied in four sympatric populations of Quercus suber (cork oak) and Q. ilex (holm oak), which form extensive woodlands with ecological and socio-economical importance in south-western Europe. Practical reasons for identification of introgressed individuals include assessing the relationship between the genetic makeup and atypical phenotypic characters, such as cork quality.

The level of introgression has been assessed using highly discriminant nuclear microsatellite loci. Bayesian clustering approaches have been used to evaluate the genetic admixture proportion at the population level and the different contributions of parental genomes to the admixed individuals. Either no introgression or low levels (up to 5%) of introgression have been found at the different locations. Such genetic results agree with the proportions of phenotype-detected hybrid individuals observed in Spanish sampling sites.

Results are discussed in relation to the state of the art of knowledge about the biogeography and the life history traits of the two species. Additionally, the efficiency of the clustering methods in identifying hybrids between the two species in our data sets was explored by means of fictitious populations with different levels of introgression, created on the basis of the observed allele frequencies in each sampling site.

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KEYWORDS:

Quercus suber, Quercus ilex, introgression, Bayesian clustering approach, nuclear microsatellites.

Spatial genetic pattern of dispersed seedlings in the California valley oak

Delphine Grivet[1], **Victoria L. Sork**[1], **Peter E. Smouse**[2]

Seed-mediated gene flow is a critical factor in local genetic structure of plant populations. While it is extremely difficult to track animal-dispersed seeds, recent innovations—the use of maternally inherited tissue and novel statistical models—provide new tools to assess the scale of seed dispersal and the impact of dispersal on genetic diversity. Here we investigate seed dispersal in Quercus lobata, a threatened California endemic oak, by analyzing acorns that are still attached to established seedlings. The combined genotypes of progeny leaf tissue and maternally-inherited seed tissue enable us to assess the impact of acorn dispersal agents on transport distances and seedling genetic diversity. The study site is the UCSB Sedgwick Reserve in the Santa Ynez valley of central California. We have previously mapped and genotyped a large portion of adult valley oak (n~300). For this study, we sampled two different settings where seedlings can be found: (i) open areas, far from conspecific adults, and (ii) beneath the canopies of seed-trees, including both acorns from that tree and those dispersed from other trees. In open areas, we sampled 300 seedlings, distributed among five patches. Beneath seed trees, we sampled 400 seedlings, distributed among 20 trees. Using a set of seven nSSR markers, we first established maternity for all sampled seeds and generated a seed dispersal curve. Second, for the five patches of dispersed seedlings, we conducted a genetic structure analysis of the seed pool to estimate the average number of effective mothers (N_{em}) per patch. Our analyses shed light on: (a) the behavioral ecology of the dispersal agents, and (b) the contribution of seed dispersal to the genetic structure of a savannah oak species, comparable to that obtained for pollen movement, available from companion studies.

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KEYWORDS:
seedling dispersal, maternity analysis, seed pool approach, seedcoat, microsatellites

Gene flow between native and exotic tree species

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The increasing use of exotic tree species in forestry and the lack of any genetic barriers within many tree genera present a risk to the genetic integrity of populations of native species. It is therefore important to monitor the rate of gene flow from plantations of exotics into nearby populations of native species. In this study we use high-throughput species-specific molecular markers (SNPstream, microsatellites and cpDNA RFLPs) to measure gene flow from plantations of exotic poplar (Populus) and larch (Larix) into natural populations of native species. For both genera, we genotyped thousands of seeds from trees of native species; samples were collected in multiple years, in order to get an estimate of the year-to-year variation in the rate of introgression. In larch on average 2% of the produced seeds had exotic fathers, with little variation between years and populations. In poplar the rate of introgression was higher and more variable, ranging from 3% to 66%, though most of the variation was between populations and mother species with little variation over the years. Overall, the found rates of introgression are high enough to have significant long-term effects on the genetic constitution of the native species.

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Measuring and modeling seed dispersal in forest trees: combining genetic and maximum likelihood approaches

Andy Jones[1] **Helene Muller-Landau**[2]

Seed dispersal is a central process in the ecological and evolutionary dynamics of forest tree populations. Regrettably, reliable estimates of seed dispersal, particularly long distance dispersal, have been difficult to obtain in closed canopy forests. Indirect methods, including inverse modeling of dispersal curves, are often used to estimate the dispersal and recruitment curves of trees, but their reliability has been difficult to assess. The advent of genetic techniques has led to direct estimates of the distances traveled by individual seeds, but because of practical limitations does not in and of itself resolve the challenge of estimating the dispersal distance distributions for the population as a whole. In this talk, I will present a comparison of estimates of dispersal distance distributions made with and without inverse modeling and with and without genetic information on genotype matches to likely parent trees for the Neotropical tree, Jacaranda copaia. Seeds were sampled from seed traps located within an 84 ha area in which all adult trees were mapped and genotyped on Barro Colorado Island, Panama. Seeds were matched to maternal parents. We developed new statistical procedures that account for sampling biases and the unavoidable censoring of data as a result of long distance dispersal for use with the genetic data. We compare the accuracy and precision of the different methods through an analysis of both empirical and simulated data. Our results suggest that inverse modeling approaches that do not incorporate genetic data are subject to much larger sampling error and thus produce much less reliable estimates of dispersal distance distributions than do those that utilize genetic matching. We also demonstrate that the distribution of genetic dispersal distances in seed traps alone can differ widely from the population dispersal distance distribution, depending on the locations of traps relative to trees and the size of the mapped area. Overall, we find that the combination of genetic identification and inverse modeling provides consistent and reliable estimates of dispersal distances, while either approach alone does not.

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KEYWORDS:

population genetics, maximum likelihood, gene flow, dispersal kernel, tropical trees

A bayesian approach to jointly estimate the pollen dispersal function and the heterogeneity of male fecundity : Application to Sorbus torminalis

Etienne K. Klein[1,2], **Nicolas Desassis**[1], **Sylvie Oddou-Muratorio**[2]

The genetic composition of the pollen cloud received by mother-trees, and subsequently the male reproductive success of the different father-trees, results from the interaction between (i) the spatial distribution of the fathers around the mothers, (ii) the pollen dispersal function, and (iii) the individual fertilities of the fathers. Disentangling the effects of the spatial pattern (of pollen sources) from that of the heterogeneity in male fecundity is a major step to understand the variation in male reproductive success. These two processes are particularly intricate for low-density species, non randomly distributed in space, with long distance dispersal.

We studied the case of Sorbus torminalis (L.) Crantz, a low density post-pioneer forest tree species, hermaphrodite and essentially self-incompatible. In a 470 ha area, all the 185 flowering individuals were genotyped at 6 microsatellite loci as well as 1073 seeds sampled on 60 mother-trees. In a previous study, we compared the TwoGener method with a mixed mating model that accounted for heterogeneity of male fecundity through three explanatory variables (flowering intensity, diameter and local density). Both methods agreed on the importance of long-distance pollen dispersal, better described by a fat-tailed dispersal function. However, the methods markedly differed in their estimates of intensity of the heterogeneity of male fecundity: the ratio between observed and estimated effective densities of pollen donor trees varied from 30 with TwoGener to 2 with our mixed mating model.

We investigated here whether this discrepancy was due to the inadequacy of TwoGener to the case of our low-density, aggregated species or to the weakness of the explanatory variables used in the mixed mating model. We developed a Bayesian approach that accounts for an heterogeneity of male fecundity within the context of the mixed mating model but without any explanatory variable. It is based on a model that considers the individual fecundity of each father-tree as a random effect. This random effect is assumed to follow a given distribution whose variance is of interest. This approach allowed us to fit to the data different families of dispersal functions as well as different distributions for individual fecundity.

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KEYWORDS:

pollen dispersal kernel ; long-distance dispersal ; mating model ; gene flow ; pollen cloud composition

Hybridization process in Mexican oaks

Ken Oyama[1]**, Antonio González-Rodríguez**[1]**, Efraín Tovar Sánchez**[2]

Mexico is one of the major center of speciation of the genus Quercus in the world with 161 species including 109 endemics. Hybridization has been proposed as one of the most plausible hypothesis to explain this oak diversification in Mexico. This work presents a summary of the documented cases of hybridization using morphological and molecular markers. We present some examples of (1) formation of species of hybrid nature (Q. xbasaseachicesis, Q. xdysophylla), and (2) patterns of within-population genetic diversity and population differentiation among hybrid species complexes (Q. laurina X Q. affinis, Q.crassipes X Q. crassifolia, Q. conzattti X Q. eduardii). We discussed the historical (e.g., climate changes in the past), geographical (e.g., distribution of hybrid zones), and ecological (e.g., colonization of new habitats by hybrids) factors that explain the patterns of morphological and genetic variation that we found. We also discuss the role of hybridization in the speciation of Mexican oaks.

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KEYWORDS:

oaks; hybridization; Mexico

A Novel Indirect Method of Estimating the Pollen Dispersal Curve that is Independent of Effective Density

Juan José Robledo-Arnuncio[1,3]**, Frédéric Austerlitz**[2] **and Peter E. Smouse**[3]

We introduce a novel indirect method of estimating the pollen dispersal curve from mother-offspring genotypic data. Unlike an earlier indirect approach (TwoGener), this method is based on a normalized measure of correlated paternity between female pairs, whose expectation does not explicitly depend on the unknown effective male population density (d_e). We investigate the statistical properties of the new method, by comparison with those of TwoGener, considering the sensitivity to reductions of d_e, relative to census density, resulting from unequal male fecundity and asynchronous flowering. Our main results are: (i) it is possible to obtain reliable estimates of the average distance of pollen dispersal, δ, from indirect methods, even under non-uniform male fecundity and variable flowering phenology, (ii) the new method yields more accurate and more precise δ-estimates than TwoGener under a wide range of sampling and flowering scenarios, and (iii) TwoGener can be used to obtain approximate d_e estimates, if needed for other purposes. Our results also show that accurately estimating the shape of the tail of the pollen dispersal function by means of indirect methods remains a very difficult challenge.

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KEYWORDS:
pollen flow, dispersal kernel, effective density, correlated paternity

Gene flow in a Mediterranean oak complex: the effects of space and time

Daniela Salvini[1], Piero Bruschi[2], Silvia Fineschi[3], Erik D. Kjær[1], Johannes Kollmann[4], Ib M. Skovgaard[5], Giovanni G. Vendramin[6]

With the objective of detecting the rate and possible directionality of natural hybridisation and to better understand the effect of the spatial distribution of trees in shaping the genetic structure of natural oak populations, we performed a fine scale study in a natural forest within a sympatric zone in central Italy. Our study species, Q. petraea (sessile oak) and Q. pubescens, (downy oak) belong to the complex Q. robur-petraea-pubescens-frainetto. Their overlapping distribution areas with frequent sympatry and the number of studies reporting the presence of morphologically intermediate trees draw our attention on the possibility of high interspecific gene flow between them. On the other hand, these species display asynchrony in their flowering times and their different ecological requirements may cause a clumped small-scale distribution. An area of 6 ha was chosen for exhaustive sampling of adult plants (295 individuals) and seedlings (387 individuals) and a subsample of 30 adult plants was chosen for seed collection (841 in total). Plant material was genotyped using five nuclear and three chloroplast microsatellite markers (SSR). Paternity analysis of seeds and parentage analysis of seedlings were carried out for the detection of pollination events. Flowering phenology and pollination biology were also studied in the same forest. Comparative approaches for the estimate of gene flow parameters were employed based on the 'neighborhood model', on the 'two-generation' analysis and on a maximum likelihood method. Both spatial distribution of trees and flowering time play an important role in shaping the direction and intensity of pollen mediated gene flow. However, long distance pollination was also detected. These results are discussed with respect to future evolution of the sessile–downy oak complex and the dynamics of their spatial genetic structure.

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KEYWORDS:

spatial genetics; flowering time; gene-flow; Quercus petraea; Quercus pubescens

I-SIII,11

Measuring Pollen Structure of *Enterolobium cyclocarpum* for a Tropical Dry Forest Landscape in Costa Rica

***Peter E Smouse*[1], James L Hamrick[2], Eva Gonzales[1], and Juan J Robledo-Arnuncio[3]**

Guanacaste (Enterolobium cyclocarpum (Jacq.) Griseb.) is a leguminous tree species of the dry tropical forests of Central and northern South America. Much of its native habitat has been cleared for pasture, but Guanacaste is a valuable shade and forage species for cattle, and is usually not cut. Still, its habitat has been steadily fragmented and opened up, which must have some effect on its pollination dynamics. We have analyzed a five-year series of pollen structure and pollen flow in four different localities in Guanacaste Province, Costa Rica, one a relatively dense dry forest (Palo Verde National Park), two heavily cleared ranches (Rancho Stewart and Hacienda Solimar), and the last a small town (Pueblo Bagaces), where remnant trees are found along the streets and in residential yards and gardens. The degree of landscape disruption increases in the order Palo Verde > Stewart ~ Solimar > Bagaces. We have assayed the genotypes of mothers and offspring (13 allozyme loci), have inferred the pollen gametic contributions of paternal donors, and have conducted a doubly-nested TwoGener analysis, estimating the among-mother & among-year/within-mother components of paternal gametic contributions. The proximal questions of the study were: (1) How different are the pollen pools of single mothers from year-to-year? (2) How different are the pollen pools of different mothers, averaged over time? (3) What does that imply about the effective number of male contributors, N_{ep}? (4) What does that imply about the mean distance of successful pollination, δ? (5) How do these answers change with progressive landscape disturbance?

A variance partition for each of the four locations indicates that while there is some year-to-year variation in pollen donors, the variation among the pollen pools sampled by different seed-parents (mothers) is larger. The fractions of among-mother (VAM), among-years/within-mother (V_{AY}), and within-year/within-mother (V_{WY}) variation are, however, different for the four sites: Palo Verde National Park (VAM = 0.091, VAY = 0.019, V_{WY} = 0.889); Rancho Stewart (V_{AM} = 0.056, V_{AY} = 0.023, V_{WY} = 0.921); Hacienda Solimar (V_{AM} = 0.054, V_{AY} = 0.024, V_{WY} = 0.922); Pueblo Bagaces (V_{AM} = 0.045, VAY = 0.022, VWY = 0.933). These results translate into pollen pool Φ-statistics. For a single year, we have $\Phi_{yt} = (V_{AY} + V_{AM}) \div (V_{WY} + V_{AY} + V_{AM}) = 0.110 > 0.079 \sim 0.078 > 0.067$ for the four sites, respectively. Over a five-year period, and allowing for year-to-year variation, we have $\Phi_{mt} = (V_{AY}) \div (V_{WY} + V_{AY} + V_{AM}) = 0.091 > 0.056 \sim 0.054 > 0.045$. We can translate the Φ_{mt}-estimates into effective numbers of pollen donors for a single seed-parent, $N_{ep} = 5.5 < 8.9 \sim 9.2 < 11.1$, respectively. Evidently, as the canopy becomes progressively more open, as a consequence of continuing landscape disruption, pollen can be expected to move more freely across the landscape. For Guanacaste, progressive landscape disturbance, at least at the level shown here, does not seem to threaten the genetic integrity of the species. [Funded by NSF-DEB-0211430]

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Spatially explicit analysis of range wide genetic structure in a California endemic tree species using chloroplast and nuclear microsatellites

Victoria L. Sork[1], **Delphine Grivet**[1]

Knowledge of historical gene flow, at the scale of a species range-wide distribution, can reveal the past processes that have shaped the current spatial genetic structure of the populations. In this study, we look at gene movements in the valley oak, Quercus lobata, a species endemic to California whose persistence is jeopardized by years of land use change and landscape alteration. We sampled ~100 individuals from 38 sites distributed throughout the range of the species and assessed seed and pollen gene flow by using chloroplast (six primers) and nuclear (seven primers) microsatellites. Levels of differentiation for chloroplast ($G_{ST} = 0.677$) and nuclear ($G_{ST} = 0.061$) genomes indicate higher gene flow through pollen than through seeds. However, the pollen to seed migration ratio for this Californian species is relatively low compared to other oak species studied so far ($r = 30$). Traditional analyses of isolation by distance did not reveal a pattern. We conducted a canonical trend surface analysis to look at the spatial structure of gene flow. The trend-surface analysis reveals a pattern of geographic variation for both sets of markers: North-South and East-West patterns characterize chloroplast markers while a North-South pattern only was observed for the nuclear markers. Thus, the chloroplast results suggest a patchy distribution that fits a gradient while the nuclear results indicate that populations are differentiated gradually across the gradient. A structure analysis of these populations based on chloroplast markers indicates that the populations can be assigned to about 15 groups, while the analysis based on nuclear markers groups the populations into a single group, probably because of the continuous and overlapping distribution nuclear alleles. These patterns provide alternative evidence that oak populations are isolated by distance and that historical gene movement has occurred through diffusion over time rather than sporadic long distance events.

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KEYWORDS:

canonical correlation, spatial genetic structure, chloroplast and nuclear microsatellites, California oak, gene flow

Fine-scale spatial genetic structure in mixed oak stands with different levels of hybridisation

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Oaks are model species for the study of natural introgressive hybridization. High interfertility among oak taxa might result in collective evolution, through interspecific spread of advantageous alleles, in this genus challenging the standard concept of species. Nine highly polimorphic microsatellite (nuSSR) loci were analysed in three mixed oak populations of Q. pyrenaica and Q. petraea (Montejo, Somosierra and Robregordo) with different density and level of hybridization. Morphological measures on leaves, jointly with nuSSR allele frequency, allowed to assess individual admixture rates. Insights about the relative effect of density and introgression rates on fine-scale spatial genetic structure (SGS) were obtained from autocorrelograms and Sp-statistic estimates based on individuals with different admixture rates. Spatial genetic structure depended on the population analysed as bigger differences, which cannot be solely attributed to census densities, were found among populations than between species. Hybridization was revealed as a potentially important factor shaping within population spatial genetic structure due to an excess of nearby interespecific matings and the spatial clustering of resultant hybrids. Indirect estimates of historical gene flow were investigated for Montejo population and compared with actual values of gene dispersal assessed by parentage analysis in a former study. Similar values were found for present and historical gene flow in both species, which might reveal a population demographical stability in the last centuries.

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KEYWORDS:
fine-scale spatial genetic structure, hybrid zones, genetic introgression, microsatellites, Quercus.

A Bayesian spatial method for inference of the geographical distribution of allele frequencies

Patrik Waldmann[1], **Mikko J. Sillanpää**[2] **and Andrew Thomas**[2]

Forest trees are often distinguished by wind pollination over large distances, outcrossing mating system, production of large number of seeds (that often have good dispersal abilities) and long generation time. The result of those biological features is that populations of many tree species often reveal very little genetic differentiation. Hence, one would also expect little spatial genetic association, and isolation by distance between tree populations only over very large geographical distances. Several studies on forest trees of nuclear and chloroplast DNA as well as allozyme markers show little spatial association.

Most studies analyzing the geographic distribution of genes are based on spatial autocorrelation techniques. These methods were pioneered by Sokal and colleagues in the 70s and 80s. The most frequently used autocorrelation coefficient is Moran's I. Plots of I estimates versus distance are denoted spatial correlograms. If spatial pattern is present, I is expected to decline with increasing distance. The properties of spatial autocorrelation techniques have been tested under different evolutionary scenarios.

Recently, there has been a remarkable increase in the use of Bayesian statistical methods in genetics. Bayesian hierarchical models for inference of allele frequencies that account for the geographical structure have been developed. These methods have quite often been applied in human epidemiology for inference of disease genes and are usually referred to as conditional autoregressive (CAR) models because of the prior that is used to incorporate the geographical information from the map.

Here we present a Bayesian spatial model that is an extension of earlier work of Vounatsou et al. (2000) and Wasser et al. (2004), but with different parameterization and testing of spatial association (model comparison) using the Deviance Information Criterion (DIC; Spiegelhalter et al. 2002). The method is implemented in WinBUGS (Spiegelhalter et al. 2005) and tested on simulated data that are intended to resemble the distribution of alleles along a cline.

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KEYWORDS:
Spatial genetics, Bayesian inference, MCMC, cline

SESSION IV

QUANTITATIVE GENETICS, QTL STUDIES AND ADAPTATION

Quercus suber geographic variation: preliminary results of the Iberian Península provenance trials

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Cork oak (Quercus suber L.) has a great social and ecological importance in the Iberian Peninsula. Yet, a large proportion of cork oak stands is old and natural regeneration is often problematic. During the last decade large areas were reforested with this species in the context of the Common Agriculture Policy (CAP) both in Portugal and Spain. Survival rates were often low due to inappropriate nursery and installation techniques, as well as to the use of unsuitable genetic material. In fact, the cork oak adaptability to environmental conditions is poorly understood; moreover climate change scenarios foreseen enhanced water deficits in the Mediterranean region. In order to allocate the most adequate seed sources to each set of climatic conditions in future afforestation activities and considering that an important source of variation in the characteristics related with adaptability may be at the provenance level, we evaluated inter-provenance variability and phenotypic plasticity for growth, survival, morphological structure and drought tolerance five years after plantation, in four test sites located across the Iberian Peninsula and covering a wide range of ecological conditions. Such trials are part of a multi-locality provenance test belonging to a Network that was bring up by Concerted action "European network for the evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies" where 35 cork oak populations covering all the natural distribution area are represented.

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KEYWORDS:

oak, genetic variability, adaptability, phenotypic plasticity

Evolution of genetic diversity and differentiation in metapopulations for genes involved in regulation networks.

Frederic Austerlitz **and Julien Guglielmini**

We develop a model that considers a quantitative trait that is coded by a set of loci, which also regulate each other through positive or negative interactions. We simulate metapopulations under either disruptive selection (strong selection for different phenotypic optima in the various populations), or convergent selection (selection for the same optimum in all populations). We study the joint evolution of the intensity of the regulations between these loci and their genetic diversity and differentiation. We show that an increasing intensity of selection yields an increased level of regulation between the loci. This is the classical canalisation process already shown in isolated populations, i.e. gene networks become more resistant to deleterious mutation through tighter regulations as the intensity of selection increases.

More importantly, we also quantify the intensity of selection to which each of these loci is subjected, by computing for each of them its within-population genetic diversity (H_S) and among-population genetic differentiation (F_{ST}). Indeed, H_S is expected to decrease and F_{ST} to increase as the intensity of disruptive selection increases. We show that the loci that answer the more to selection are the one that have evolved the stronger regulatory action on the other genes. This has been observed in natural populations; for instance the FRIGIDA gene, which is a regulatory gene involved in the control of flowering time in Arabidopsis thaliana, shows quite a high F_{ST} value. Our results have important consequences in the context of the search of genes under selection through genome scans. Indeed, we show here that the genes that are the more likely to be detected under this framework are the more regulatory ones.

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KEYWORDS:

gene networks, disruptive selection, quantitative traits, interactions, epistasis.

Genetic variation of spring cold hardiness in Spanish wild populations of *Castanea sativa*

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Castanea sativa Mill (Franco) is considered a noble hardwood due to the high price of its wood, which is used, among others, for furniture and veneer. Resistance to spring frost is directly related to other traits, such as apical dominance and stem form, so the study of the spring frost resistance is then important. Moreover, the climatic change is producing disarranges in the climate, which may increase late frost events during or after bud burst. The study of the intra-species variation in adaptation to frost events becomes essential. The objective of the present study was to evaluate the variability of Spanish chestnut populations in susceptibility to late spring frosts. Six wild populations from six distant sites, representing the wide Spanish distribution area of C. sativa, were selected. Four sites were selected in the Northern region, one site in the Central region and another one in the extreme Southern area of the natural distribution. Main climatic differences among sampled populations were summer drought and continental character. Genetic variation in spring cold hardiness of shoot cuttings of these six C. sativa Spanish populations was studied prior to bud burst in March 2006. Four twigs from 15 trees per family from a total of 41 families (5-8 families per population) were collected in a provenance-progeny test. These twigs were subjected to artificial freezing under controlled environmental conditions in a freezing chamber. They were tested at four freezing temperatures. Damage on each twig was recorded as visible injury to bud and stem tissues separately. Bud burst stage for each shoot cutting was also recorded before the assay. Differences among populations were highly significant for all traits and with clearly higher variation among populations than within populations. In particular, differences within populations were not significant for the stem tissue damage. The Northern populations coming from a humid climate were more resistant than the others. Phenotypic correlations among different injury traits were very high, which means that any injury trait can be used for screening spring frost resistance. Phenotypic correlations were also moderate and highly significant among injury traits and bud burst. These relationships were also apparent when analysing different injury damage data using bud burst as covariate. Highly significant differences were still found among populations in this reanalysis, suggesting that other genetically controlled factors can be involved in spring frost genetic resistance.

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KEYWORDS:
Chestnut, spring frost, wild population, genetic differentiation, climate change.

Temporal monitoring of autumn gene expression within and among populations of Sitka spruce reveals candidate genes for adaptation to local climate

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Adaptation to winter cold by conifers involves extensive physiological remodeling, the timing and extent of which varies greatly along latitudinal gradients for a variety of tree species. Studies of cold tolerance in Arabidopsis thaliana suggest that autumn cold acclimation in conifers will correspond to substantial changes in gene expression. However, fundamental differences exist between transient increases in cold tolerance in response to sudden stress in annuals such as Arabidopsis, and seasonal cold acclimation in conifers. In addition, the extent to which among-population phenotypic variation in cold hardiness in forest trees corresponds to differences in gene expression is unknown. We have undertaken a transcript profiling study through the fall hardening period in Sitka spruce (Picea sitchensis), using a 22K Picea cDNA microarray. Four-year-old seedlings originating from a population at the core of the species range (Prince Rupert, British Columbia) were sampled from a common garden in Vancouver, for RNA extraction at five timepoints between August and December, 2004. To assess cold hardiness, each individual, on each sampling date, was artificially freeze-tested. High levels of among-population differentiation for Sitka spruce cold hardiness-related traits have been demonstrated previously. Therefore, in addition to expression profiling across all five timepoints in the core population, we have conducted among-population hybridizations between individuals originating from the north and southern limits of the species range (southern Alaska and northern California, respectively). Results from this study reveal the complexity of the transcriptional reorganization that occurs during cold acclimation in conifers. In addition, intriguing differences were observed among northern, central and southern populations in terms of the timing, extent and constitution of their respective autumn gene expression programmes. Our data suggest that phenotypic divergence in Sitka spruce cold tolerance may in part be explained by variation in patterns of gene expression. The ultimate goal of this project is to identify nucleotide variation that correlates with observed phenotypic variation. As such, cold hardiness candidate genes identified as upregulated by the microarray will be surveyed for nucleotide variation in the form of single nucleotide polymorphisms (SNPs), and correlations will be sought between SNP variants and the cold hardiness phenotype in an association study.

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Adaptive response to climate change: warming-linked spatial and temporal trends in gene frequency in Fagus sylvatica

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Rapid increases in global temperature are likely to impose strong directional selection on many plant populations. In the fragmented habitats that result from the activities of humankind, migration of some tree species may lag behind changes in climate, forcing trees to adapt if they are to survive. Within populations, microgeographic genetic differentiation of individuals can occur with respect to climatic and edaphic factors. Some tree populations may therefore adapt to changing temperatures in the short term through rapid changes in gene frequency due to differential survival of trees according to climate during their establishment from the seed pool. We used a genome scan of Amplified Fragment Length Polymorphism (AFLP) data to identify temperature-related adaptive differentiation of individuals of the tree species Fagus sylvatica within a natural forest population. We identified a locus at which populations were exceptionally differentiated in comparison with their differentiation throughout the F. sylvatica genome. By combining AFLP and dendrochronological data, we assessed spatial and temporal variation in gene frequency at this locus. We show that gene frequency varies predictably with temperature. The probability of the presence of the dominant marker allele shows a declining trend over the latter half of the 20th Century in parallel with rising temperatures in the region. Our results show that F. sylvatica populations may show some capacity for an in situ adaptive response to climate change. However as reported ongoing distributional changes demonstrate, this response is not enough to allow all populations of this species to persist in all of its current locations.

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KEYWORDS:
adaptation, climate change, genome scan, natural selection, population genomics

Migration, selection and clines in adaptive variation in *Pinus sylvestris*

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Many life history traits in forest tree species exhibit clines along environmental gradients. The timing of bud flush and bud set are typical examples. Possible explanations for this phenomenon lie in the demographic histories of the species and/or more likely, migration and locally differing selection. Here, our goal is to investigate the forces that maintain the variation seen in the timing of bud set of Scots pine (Pinus sylvestris) along a latitudinal gradient.

In a greenhouse experiment conducted as a part of the TREESNIPS project, the time from seed sowing to bud set at the end of the first growing season in 15 populations showed a linear cline along latitude (R^2=0.94), with time to bud set ranging between 136 days in a Spanish population to 90 days in a Northern Finnish population. The population-specific heritabilities were between 0.3 and 0.9.

We evaluated the role of selection in the maintenance of the cline by obtaining fitness estimates from relative survival and tree height observed in long-term provenance transfer trials. We attempted to estimate migration in terms of gene flow mediated by pollen and seed dispersal based on empirical results already published in the literature and our own data. Finally, we tried to connect these results to theoretical models of clines in polygenic traits. Specifically, we address the questions of migration-selection balance, local adaptation and genetic architecture underlying the phenotypic trait.

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KEYWORDS:

migration-selection balance; clines; gene flow; fitness; local adaptation.

An integrated approach to bud set in poplar: phenotypes, candidate genes, and QTLs

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The seasonal cycle of growth and dormancy is a distinct character of perennial plants and represents one of the most basic adaptations of trees to their environment. The recurrent transitions of meristems into and out of dormancy are of primary significance to plant productivity and survival. These transitions are tightly linked to the yearly dates of bud flush and bud set that in turn delimit the growth season.

In poplar, perception of a short-day signal leads to apical bud set and dormancy induction. Because bud set is of prime importance in determining season length in a changing environment, we dissected this developmental program at the phenotypical, genetical and molecular levels. Taking advantage of the poplar genome sequence, we integrate candidate genes obtained in large-scale gene expression studies with information of QTLs determined in three poplar pedigrees.

Expressional candidate genes were identified in the apex/bud during short-day-induced bud set, using cDNA-AFLP and micro-arrays. Apical buds of plants were sampled at weekly intervals during six weeks of short days, after which apical buds are fully dormant and cannot resume growth without prior cold treatment. The set of significantly differentially expressed genes comprises 1350 genes.

For phenotypic measurements in the field, a new bud-set scoring system was developed: bud set is phenotyped with seven developmentally distinct stages. This system allows accounting for the onset of the bud-set process as well as for its dynamics, as opposed to most earlier studies that rely on the date of accomplished bud set only. Applying this system, high-resolution phenotypic data were obtained in three poplar pedigrees, grown in two environments, and a collection of 450 Populus nigra accessions.

QTLs for different bud-set-related traits have been determined in the three pedigrees and have been positioned onto the poplar genome sequence. Integrated with the position of the expressional candidate genes, they identify genomic regions of relevance for bud set.

Together, this integrated approach provides a comprehensive overview of bud set in terms of the molecular processes associated with aspects of bud development and elucidates the genetic architecture of bud set. Genes or genomic regions identified through this combined approach are tested for their adaptive relevance in association genetics approaches (see abstract of Zaina et al.).

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Why Are Some Leaves Big? Linking Transcript, Qtl And Ld Approaches To Understand The Genetic Control Of Leaf Size And Shape In Populus.

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In poplar trees, leaf size is linked to fast growth and the production of increased wood as biomass with fast growing trees characterised by large leaves that can intercept optimum amounts of photosynthetically active radiation. Leaf size and shape are adaptive traits known to vary with environment, for example in response to temperature and light environment, but for trees little is understood on the genetic determination of such adaptations. Here we wish to understand the genetic determination of leaf size and shape in Populus.

Using a mapping population (family 331) in several different experiments over a period of five years, we have identified several QTL that occur consistently for leaf size, leaf shape and their cellular determinants (cell area and cell number). These QTL have allowed us to develop a list of candidates genes from the physical sequence of P. trichocarpa now available on-line, for our further study.

Complementary to this, we have used a large natural population of P. nigra (black poplar) from different latitudes across Europe and grown approximately 500 genotypes at one site in Belgium. Each genotype has been assessed for leaf size and shape and clear phenotypic differences were identified, for example with a cline from SW to NE Europe with leaf size increasing along this cline. Extreme populations and individuals within populations were identified for leaf size with large and small leaf genotypes originating from the NE and SW of Europe, respectively.

Transcript analysis on the small leaved and large leaved genotypes has again revealed a group of genes for further study that have been assessed relative to the positioning of QTL. SNPs detection and genotyping in this large population should enable us to use an association mapping approach to provide a final link between leaf size and shape and the underlying genetic determination of these traits.

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An example of an integrated approach to assess the genetic and adaptive variation in populations of *Castanea sativa* Mill.

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The capability of forest tree populations/species to respond to changing environmental selective pressures is largely dependent on their long-term evolutionary potential, in terms of functional gene mutations. Significant knowledge on the amount of variation available in extant genetic stocks and accurate measures of additive variance of adaptive traits are fundamental prerequisite to predict the adaptability of those population/species to the future environmental change. Addressing the above issues should provide the basic criteria for both improving the formulation of appropriate conservation strategies and for the identification of suitable seed sources regarding breeding purposes. The wide distribution of chestnut throughout Europe (particularly in the Mediterranean region) exposes this species to a variety of environmental conditions. Its expansion occurred under the selective forces of a changing environment and, especially during the past two millennia, under the human influence. Such a capacity of dynamic colonisation in a species has been attributed to its significant potential for adaptability. Genetic variation based on molecular markers as well as variability in bud burst, juvenile growth and drought tolerance was evaluated in populations of Castanea sativa Mill. spanning the European distribution range. Furthermore estimates of genetic control as well as genotype x environment interactions for the above traits were obtained by comparing progenies grown at different temperature/humidity regimes both in controlled environment (growth chamber) as well as in comparative field trials. Finally the genetic basis of such adaptive variation was established through the construction of a QTL map. Evaluation of intra and inter population genetic variation was based on 82 populations analysed by isozyme, ISSR and SSR molecular markers. Phenotypic evaluation was carried out on open pollinated families from six populations coming from contrasting sites with respect to their xerothermic index value. QTL map was based on a F1 progeny (200 individuals) obtained from a controlled cross between two trees from contrasting sites.

High genetic variation was detected among the investigated populations and major European gene pools were identified. Significant phenotypic variation was detected at all studied traits and positive correlation between phenotypic plasticity for the drought tolerance and xerothermic index values of the sites of origin was identified. Candidate genes for the adaptive traits examined were identified and mapped. Such a study provides the basic information required to predict how chestnut populations will cope with expected climate changes and for the formulation of appropriate conservation priorities.

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KEYWORDS:
Castanea sativa, population genetics, adaptive variation, QTL, gene conservation

WORKSHOP - A

ASSOCIATION MAPPING IN FOREST TREES

Association genetics for wood property traits in *Pinus taeda*

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Genetic association is a powerful method to dissect complex adaptive traits due to i) fine-scale mapping resulting from historical recombination; ii) wide coverage of phenotypic and genotypic variation within a single experiment; and iii) the simultaneous discovery of loci and alleles. In this paper, genetic association between single nucleotide polymorphisms (58 SNPs) from 20 wood- and drought- related candidate genes and an array of wood property traits with evolutionary and commercial importance, namely earlywood and latewood specific gravity, percentage of latewood, earlywood microfibril angle and wood chemistry (lignin and cellulose content), was tested using general (GLMs) and mixed (MLMs) linear models. Population structure, a common systematic bias in association studies, was assessed using 22 nuclear microsatellites. Several phenotype:genotype associations were found, some of them confirming previous evidence from collocation of QTL and genes in linkage maps, and two that involve nonsynonymous polymorphisms. The strongest genetic association found in this study was between allelic variation in α-tubulin, a gene involved in the formation of cortical microtubules, and earlywood microfibril angle. Intragenic LD decays rapidly in conifers, thus SNPs showing genetic association are likely located in close proximity to the causative polymorphisms. Candidate gene strategies to dissect complex adaptive traits are of particular utility in species, such as conifers, where genome-wide strategies are limited by their large genomes.

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KEYWORDS:
association genetics; candidate genes; microfibril angle; SNPs; Pinus taeda

ADEPT1 – Association genetics for disease and drought traits in loblolly pine

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Approaches to dissect complex traits based on candidate genes are appealing for conifers because of their large and highly-duplicated genomes. Drought and fungus diseases are important causes of mortality and yield losses in loblolly pine (Pinus taeda), a major source of softwood in the World. In the framework of ADEPT1 project (Allele Discovery for Genes Controlling Economic Traits in Loblolly Pine), diversity and divergence DNA sequence data over 50 stress-related candidate gene loci were generated, including several genes functionally involved in drought (e.g. dehydrins) and disease (e.g. PR genes) response. For drought, only two candidate genes showed a pattern of polymorphism that did not agree with neutral patters, erd3 and ccoaomt-1; the latter, a methylating enzyme involved in lignification, showed dimorphism (i.e., two highly divergent haplotype lineages at equal frequency), which is commonly associated with the long-term action of balancing selection. For disease, polymorphism patterns concordant with both trench-warfare (e.g. erebp) and arms-race (e.g. myb3) hypotheses were found in several loci. From 40 stress-response candidate genes, SNPs were selected based on their frequency, putative influence on protein structure, and patterns of linkage disequilibrium within candidate gene loci and were genotyped with a FP-TDI SNP-typing platform, over a population of 960 individuals selected from FBRC-Family Based Association Population. Quantitative Transmission Disequilibrium Tests, which accounts for family structure, and a novel likelihood method were used to detect genetic association between SNP genotypes and (i) isotope discrimination phenotypes (i.e. related to water-use efficiency) and (ii) three quantitative (pitch canker lesion length, and fusiform rust gall length and width) and one qualitative (fusiform rust gall score) fungal disease resistance traits, in different testing sites. A total of five, nine and four SNPs were identified to be associated with isotope discrimination, pitch canker phenotypes and fusiform rust tolerance, respectively. Our study has shown the feasibility of candidate gene strategies to dissect complex adaptive traits in forest trees, provided that genes belonging to key pathways and appropriate statistical tools are used.

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KEYWORDS:
candidate genes; genetic association; isotope discrimination; disease tolerance; Pinus taeda

DEPT2 – Re-sequencing and SNP Discovery in Loblolly Pine

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The ADEPT2 project is aimed at determining the associations between natural genetic variation and complex traits (http://dendrome.ucdavis.edu/adept2). This is a collaborative project between UC Davis, North Carolina State University, University of Florida and Texas A&M University. Each partner is taking the lead on a part of the overall project and UC Davis is conducting all SNP discovery and genotyping. This will then be combined with the phenotyping being done at the partner institutes for analysis. The re-sequencing of 10,000 genes in loblolly pine in a diversity panel of 18 individuals is underway. Primers have been designed by Agencourt Biosciences for all EST clusters identified from the University of Georgia loblolly EST libraries and are in the process of being validated. Haploid megagametophyte tissue is being used for the re-sequencing and has been extracted and sent to Agencourt. Re-sequencing has begun and as sequences are received from Agencourt they are sent through a SNP discovery pipeline developed at UC Davis that is based on the polybayes SNP identification program. All information about the DNA extractions, trees, ESTs, primers, validation, sequences and SNPs are being entered into the TreeGenes database to ensure data integrity and allow users to track data. This information will be available through a series of interfaces developed at UC Davis. SNPs will then be evaluated and a subset used to genotype three loblolly pine populations for which phenotype data is available or is being collected from five phenotypic groups, wood properties (UF), disease resistance (UF), drought resistance (NCSU), gene expression (TAMU) and metabolomics (UCD). From here we will have the foundation for looking into the diversity of the loblolly pine genome and dissecting association between genotypes and phenotypes. At UC Davis we will develop the tools to give the community access to the data required for more detailed analysis.

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Report on the progress in the pine family comparative genomics project

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The Conifer Forest Health Genomics project (2006-2009) funded by Genome B.C. and Genome Canada follows the successful Treenomix project and uses the tools previously developed within its predecessor. Conifer health in British Columbia is seriously threatened by pest infestations like the white pine weevil that cause enormous economic losses in forestry each year. The urgent need to breed sufficiently resistant populations leads to the joint effort between our functional and our comparative genomics research groups to investigate the genetic resistance trait in more detail. We present here the elements and the ongoing progress for the comparative genomics program: (1) SNP discovery in candidate genes, (2) association genetics and QTL mapping, (3) establishment of syntenic genetic linkage maps. We assay a panel of reverse transcribed mRNA using 24 individuals throughout the range of white, Engelmann and Sitka spruce to identify SNPs in 1500 loci in spruce. Bioinformatics will allow us to identify polymorphisms of likely functional importance. Plant material for association and QTL studies has been harvested at the beginning of this year's growing season. For a progeny of approximately 1000 individuals from the breeding population (B.C. Ministry of Forests) we will do association studies based on the candidate SNPs. One of our pilot projects for this year's research program is the genetical genomics approach to uncover the genetic basis of constitutive resistance in spruce. Expression and metabolite QTL mapping will be conducted by using complete diallelic crossing designs (six 75 member crosses) derived from the B.C. Ministry of Forests full sib weevil progeny tests. As much as 384 candidate genes will be used in a high-throughput genotyping effort to establish framework linkage maps for QTL mapping. Conserved ortholog set (COS) markers as identified from our inhouse EST databases (spruce versus pine) allow the construction of syntenic maps. So far half of the 453 tested COS primers amplified single products in both white spruce and loblolly pine. The eventual goal derived from the existence of synteny is thus the transfer of QTLs among members of the pine family.

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KEYWORDS:
conifer health; candidate genes; SNP discovery; association and QTL mapping; syntenic maps.

Association genetics projects in European forest tree species

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Following the recent developments of nucleotide diversity studies in genes potentially involved in adaptive traits (i.e. traits of interest in the context of forest tree species responses to global change: drought tolerance, bud burst), association studies are currently underway in a number of European forest tree species, which aim at testing and validating identified SNPs putative functional roles on phenotypic variation in the traits of interest. Most of these studies deal with outcrossing species, large population size natural populations, SNPs from limited number of sites from a limited number of genes but aimed to be genotyped on large progeny samples from representative populations, and finally with already observed and predicted very small linkage disequilibrium windows within those genes. After an overview of some current European projects and the presentation of preliminary results, the consequences of the specific features characterising the forest tree species involved on the efficiency of future association studies will be discussed. These projects could serve as a tremendous springboard for improving at the same time 1) the choice of genomic regions targeted in the prerequisite and essential nucleotide diversity step of the projects, and also 2) the methodological and statistical aspects of applying association mapping models, testing in particular new Bayesian models which seem to better fit the candidate genes - based approaches currently used.

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Association genetics for wood-, drought- and photoperiod- related candidate genes in maritime pine (Pinus pinaster Aiton)

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Association genetics is a relatively new approach for the dissection of complex quantitative traits. By using natural populations, association genetics techniques take advantage of natural variation and several generations of recombination to identify (and quantify) genotype-phenotype associations at a finer scale than classical QTL mapping. In the framework of the TREESNIPS project, we screened SNP variation (over 45 SNPs from 14 candidate genes related to drought tolerance, wood physical properties and photoperiod) in an association population of over 500 trees, covering the full-range of the species. Different quantitative traits, including isotope discrimination ratios (related to water use efficiency) but also growth traits and biomass, were measured. A set of eight nuclear microsatellites was used to compute a Q matrix; then a structured association analysis based on logistic regression ratio tests was performed. Microsatellites showed a clear population structure in the native range of maritime pine, with different groups of populations: Continental France, Iberian Peninsula, Corsica and northern Africa. Different genotype:phenotype associations were found and their reliability and use in conservation and breeding of maritime pine genetic resources will be discussed.

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KEYWORDS:
adaptation; association mapping; isotope discrimination; SNPs; Pinus pinaster

A SNP-based genetic map of expressed regulatory genes in the conifer white spruce

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The objective of this study was to map several hundreds of expressed genes coding for transcription factors (TFs) in the conifer white spruce (Picea glauca), in an effort to develop a high throughput gene-based genotyping assay, and to better comprehend the organization of regulatory genes and gene families in the conifer genome. More than a thousand single nucleotide polymorphisms (SNPs) of expressed genes were identified as candidates for mapping by resequencing from genomic DNA. A total of 768 SNPs representative of 425 distinct genes were used to construct an Illumina bead array, a highly multiplexed SNP genotyping assay. Out of these, 38 SNPs were detected in silico from the analysis of EST collections without resequencing. SNP calls were made for two parents and their 292 progeny derived from an outbred F_1 cross. The reproducibility of the SNP assay was tested with five replicates of each parent and the accuracy rate was over 99%. SNPs identified from resequencing for each parent could be mapped at a rate of 70% among the progeny. In silico predicted SNPs could be mapped at a lower rate of 29%. A number of ESTP and SSR markers genotyped for the same cross in a previous study were added to the SNPs. Altogether, they were used to construct individual linkage maps and to assemble a composite map using JoinMap v.3.0. The composite map had a length of 1790.1 cM (Kosambi) and contained a total of 349 positioned markers representative of as many distinct gene loci, including 307 SNPs. These markers were assembled into 12 major linkage groups. The distribution of genes and gene families among the linkage groups will be presented and discussed.

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KEYWORDS:

Gene duplication, genome mapping, HT genotyping, Illumina, Picea glauca, regulatory genes, SNPs, structural genomics.

Association genetics for adaptive traits in Douglas-fir

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Adaptation is critical to plant survival and growth in a changing environment. The genetics of plant response to cold and drought stress, and to changing photoperiod is well understood and recent microarray studies in model organisms are beginning to elucidate putative candidate genes for the control of these responses. In trees, size and complexity of the genome precludes any attempts at positional cloning and yet, understanding the molecular basis of adaptation is in these long-lived species is critical to improving tree-breeding and conservation efforts, and to understand the impact of global warming on forests in the US. We are using candidate gene-based association mapping to identify the loci and alleles responsible for phenotypic variation among conifers. We have already successfully demonstrated this approach and identified SNPs associated with variation in wood quality and response to drought stress in loblolly pine (Pinus taeda L.). Our research in Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) aims to identify variation in candidate gene homologs for cold tolerance and test these for association with collected phenotypic data in a population of c.900 trees which have been tested for evidence of population structure using both isozyme and SSR markers. The extensive range of this species across the heterogenous environments of the western US demonstrates its remarkable capacity for adaption. We report the identification of homologous sequences representing 100 candidate genes for low temperature tolerance based on a modest EST collection and the success and efficacy of automated primer design, sequence validation and SNP identification within these sequences.

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Sylleptic branching as an adaptive trait in Populus – QTL, candidate genes and association genetics.

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Sylleptic branches, which grow from lateral buds during the same growing season, are known to play an important role in the generation of biomass in poplars through increased leaf area enabling the capture of more light and greater surface area for gaseous exchange. Sylleptic production may also be considered as an adaptive trait, being strongly influenced by environment. Control of sylleptic branch production has been hypothesised to be under the control of a number of genes, therefore a quantitative genetic approach is necessary to unravel the control of this trait.

At present there are no definitive genes identified as being involved in initiation, growth and termination of sylleptic branches, and reasons for variation in their production between or within different species is largely unknown, although environment has been shown to have a role. This study aims to understand the control of sylleptic branching, and the traits adaptation to the environment using multiple approaches such as QTL mapping, hormone profiling and a candidate gene approach.

As part of th POPYOMICS project, an F_2 pedigree (Family 331; POP1) derived from a cross between Populus trichocarpa (93-968) and P. deltoides (Ill-129) was grown at three geographically different sites across Europe. These trials have established that the average number of sylleptics produced by P. trichocarpa was nearly six times (5.7) greater than for P. deltoides. The F_1 parents showed intermediate values and F_2 shows normal distribution with evidence of transgressive segregation. Significant genotype x environment interactions were seen despite heritability values being moderate to high. Quantitative Trait Loci (QTL) were identified across the three sites. Additive genetic effects of QTL were seen to differ across sites for sylleptic branch trait QTL.

The pedigree was also grown under elevated CO_2 conditions which was seen to increase sylleptic branch production. QTL for response to CO_2 concentration were identified.

Hormone assessments showed levels to vary between the grandparent species which may go some way towards explaining the lack of control observed in P. trichocarpa on sylleptic branch production. Candidate genes seen to collocate to regions where QTL mapped were identified.

The study of a natural population of P. nigra has shown variation in number of sylleptic branches to be highly significant across regions and may allow for a candidate gene approach to association mapping to be carried out.

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Using an association (linkage disequilibrium) mapping approach for phenological traits in *Populus nigra*

***Giusi Zaina*[1], Antje Rohde[2], Marijke Steenackers[3], Wout Boerjan[2], Michele Morgante[1]**

Association mapping, based on linkage disequilibrium (LD), offers an alternative method for mapping quantitative/adaptive traits in tree species, using ancestral recombination events in natural populations to make marker-phenotype associations. These mapping studies depend critically on the extent of linkage disequilibrium between functional alleles and surrounding single nucleotide polymorphism (SNPs) markers, since the LD extent affects the number of markers that should be genotyped in the population.

Taking benefit of the recent release of the genome sequence of Populus trichocarpa, we analysed sequence diversity and the linkage disequilibrium structure in a European poplar species, Populus nigra. Nucleotide diversity estimates were comparable to those of other poplar species and forest trees, but significantly lower than in Populus tremula. Polymorphism survey across four large genomic regions showed that the levels of polymorphisms are not limiting for association studies and LD estimates are low but significant within few kbs. Those results suggested that LD in poplar is not extensive enough for a whole genome association approach, but is suitable for the candidate gene approach, since LD blocks span one or a few genes limiting the risk of false positive associations. Hence, we identified a set of candidate genes for phenology based on the literature on flowering time pathway in Arabidopsis thaliana and we also identified SNPs within them to be used as markers for the association.

An association population of about 400 P. nigra genotypes, collected along 10 degree's latitude throughout Europe, was established in a common garden experiment. Among others, bud set phenotypes were scored in the population and then considered for the association analysis with the set of SNPs genotyped. Despite the adaptive population differentiation of the bud set traits (Qst of 0.6), we observed low-to-moderate levels of genetic differentiation (Fst from 0.05 to 0.1) in the candidate genes analysed. We also found significant associations between few candidate gene polymorphisms and bud set phenotypes.

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KEY WORDS:
Populus; genetic diversity; linkage disequilibrium; association mapping; bud set.

WORKSHOP - B

SIMULATION MODELS OF TREE POPULATION GENETICS

AMELIE - A simulation model linking spatio-temporal population-genotype dynamics of trees

Anna Kuparinen **(presenting author), Frank M. Schurr**

Simulation models are needed to describe population-genotype dynamics of trees with a sufficient level of realism. We formulate a grid-based simulation model, AMELIE, that links the spatio-temporal dynamics of plant populations and genotypes. AMELIE is flexible in the description of life histories, competition among individuals, reproductive systems and demographic and environmental stochasticity. It can incorporate alternative submodels for key demographic processes (including arbitrary pollen and seed dispersal kernels). Genotypes of trees are characterized by the combination of two alleles at a bi-parentally inherited locus that may affect any demographic process.We use AMELIE to study how transgene spread from a genetically modified (GM) to a conventional forest depends on the demographic trait modified by the transgene, on transgene expression, and on the initial genotype of GM plants. Transgene spread strongly depends on the interplay between trait modification and transgene expression, which jointly define the ability of heterozygous trees to replace conventional ones. When examining two alternative measures for controlling transgene spread from a GM tree population, we find that planting heterozygous GM trees initially reduces transgene spread, whereas transgene recessiveness has more long-term effects on gene flow.The results of this study have direct implications for the assessment and management of the risks related to the plantation of GM forest. The flexible simulation model for population-genotype dynamics furthermore has various applications in conservation biology.

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KEYWORDS:

population-genotype dynamics, gene flow, transgene spread, invasion

Ecological factors shaping the genetic quality of seeds and seedlings in forest trees: a simulation study coupled with sensitivity analyses.

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Natural regeneration is a key process in the life cycle of populations within ecosystems. Indeed, the demographic structure and genetic make-up of the adult population is mostly determined by pollen and seed dispersal, germination and seedlings mortality, in particular for long lived organisms such as forest trees. The present study aims at disentangling the effects of various ecological processes on the genetic quality of seedlings in contrasting regeneration contexts. We develop an individual-based, mechanistic model to simulate the regeneration process as the result of adult tree spatial structure (density, aggregation), mating system (shape and range of the pollen and seed dispersal curves, annual/individual male and female fertility variations) and inbreeding depression (through mortality and growth rates). We use sensitivity analyses of the model output (genetic diversity and structure of the seedlings) to identify the most influential ecological factors, and to detect interaction effects between them. These results are then confronted to observations in two different ecological contexts: one where regeneration is supposed to be gradual in a closed canopy forest environment (Cedrus atlantica and Abies alba species) and one where regeneration occurred over a short period after a severe wind storm that fell most adult trees (Fagus sylvatica and Pinus sylvestris species). Our study will provide valuable insights for multi-generation modeling in ecological genetics, and also for the sustainable management of natural forest ecosystems.

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KEYWORDS:
simulation model, natural regeneration, sensitivity analyses, CAPSIS, Morris design.

Coalescent simulations for the evaluation of statistical tools for demographic inference and the effect of homoplasy in chloroplast microsatellites

Miguel Navascués[1, 2] & Brent C. Emerson[1]

Chloroplast microsatellites, or simple sequence repeats (cpSSRs), are becoming increasingly popular markers for population genetic studies in plants, especially in conifers. However, there has been little focus on their potential for demographic inference. We have explored the utility of haploid fully linked microsatellies (i.e. chloroplast microsatellites) for the study of population expansions. We have investigated the power of current methods (mismatch distribution analysis and the F_S test) applied to cpSSRs data with coalescent simulations of different demographic scenarios. These methods are based on unrealistic mutation models where no recurrent or back mutations (i.e. homoplasy) occur and, therefore, genetic distances and diversity can be estimated without error. However, the application of these models to empirical data might affect the power of the tests particularly for microsatellite markers which evolve in a stepwise fashion and high levels of homoplasy are expected. The use of computer simulations allows tracking every homoplasious mutation in the evolution of the populations that would be hidden in an empirical study. We found that cpSSRs are sensitive to population expansion but a reduction of the power of the analysis for simulations was associated to high levels of homoplasy. Our results suggest that developing statistics based on more realistic mutational models might improve the usefulness of this type of markers in demographic inference.

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KEYWORDS:

simulation, population expansion, homoplasy, chloroplast microsatellites, coalescent

Demographic history of Pinus sylvestris inferred from nucleotide diversity data

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Past climate changes, glacials and interglacials have affected distributions of most European tree species. Some tree species have survived the changing conditions by shifting their ranges according the changing climate. Others have only existed in refugia - in small isolated regions, which have had more tolerable conditions than surrounding regions. Most temperate European forest tree species have survived the last Ice Age in Mediterranean refugia. Scots pine, however, might have different history due to its cold tolerance. Fossil data suggests that Scots pine has been abundant in Central and Western Europe during the glacial period, but has existed in lower densities during the Eemian warm period (130 000 – 110 000).The aims of the study were to examine the demographic history of Pinus sylvestris during the last glacial period (110 000 – 20 000 years ago) and interglacials, and to find out how the cold and warm periods have affected its population size and distribution. To study the question from genetic data, 16 nuclear loci were sequenced from 40 individuals from different parts of Europe (Northern Europe, Central Europe, Spain and Turkey). Hudson's ms program was used to simulate data under different demographic scenarios and summary statistics of the simulated dataset were compared to the summary statistics of the observed data.Central and Northern groups had significantly negative Tajimas's D, but in the Spain and Turkey Tajima's D was slightly positive. Fay and Wu's H was negative in all four groups. Some of these patterns could be caused by selection, but we consider demography as a more plausible explanation, since the pattern is observed across populations and across loci. Results suggest that Northern and Central European populations share the same history, but those demographic events cannot explain the pattern of nucleotide diversity in Turkey and Spain. Based on simulations, it seems that bottlenecks, rather than constant population or growth models could explain the observed pattern of nucleotide diversity in all populations.

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KEYWORDS:
Pinus sylvestris, demographic history, coalescent, nucleotide diversity, glacial history

Spatial distribution of tree species in tropical forests under a neutral hypothesis

Olivier J. Hardy[1] **& Bonaventure Sonké**[2]

Small scale spatial patterns of tree species distribution is expected to depend mostly on 1. the spatial heterogeneity of environmental conditions, 2. the modes and patterns of seed dispersal. When the effect of environmental heterogeneity can be neglected, species with limited seed dispersal are expected to aggregate more than species with extensive dispersal abilities, so that dispersal distances should be inferable from the spatial distribution of the individuals of each species. Adapting theoretical results of isolation by distance models from population genetics, we can describe what is the expected pattern of species distribution according to seed dispersal distances, assuming a neutral hypothesis (all individuals are competitively equivalent, regardless of the species) and independency of dispersal events. Simulations show that estimates of seed dispersal distances can then be obtained. The spatial patterns of species distribution was investigated in a tropical forest of Cameroon and seem to conform to the predicted patterns for most species. For some species, a significant impact of environmental heterogeneity could also be detected.

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KEYWORDS:

species distribution, isolation by distance, modeling, seed dispersal, tropical trees.

Indirect estimation of long-distance dispersal characteristics using spatially explicit individual-based simulation of metapopulation dynamics in a linearly dispersed plant.

Allan Strand[1]

Dispersal events, particularly the long-distance events important in processes such as colonization, are notoriously difficult to observe directly. As a result, neutral genetic markers have been used extensively to infer among-population dispersal, often based upon Wright's relationship between Nem and FST. This approach has rightly received much criticism in recent years, mainly due to assumptions about island population structure, equilibrium population size, mutation model and statistical behavior of Nem. Furthermore, estimates of dispersal based solely on genetics only characterize effective, rather than actual, dispersal. In this study I estimate actual dispersal indirectly through simulation of realistic demography and population genetics for an abiotically dispersed strand plant, Cakile edentula, in the southeastern US. Simulations employ a spatially explicit and individual-based population genetic model to simulate both within and among-population dynamics. Seed dispersal within and among populations is a mixture of short and long-distance distributions. The proportion of seeds emigrating from sites was empirically estimated based upon mark-recapture. Survivorship and fecundity probabilities within populations are specified using an empirically calibrated stage-based model. The mean of the distribution governing long-distance dispersal is manipulated until simulated population-genetic subdivision equals the empirical estimates obtained from analysis of neutral genetic markers. Unlike estimates of dispersal based upon equilibrium assumptions, this analysis also includes extinction-recolonization dynamics where extinction rates are estimated from historical tropical storm records. For this project I have extended rmetasim (http://cran.r-project.org) by including spatial coordinates for each individual and implementing 2d dispersal kernels for both seed and pollen. This software can be installed on most computers as a dynamically loaded package in R. It is able to model any metapopulation dynamics and population genetics with stage-based within-population demography. This work demonstrates one way that individual-based simulations of population genetic processes provide a natural tool to integrate non-equilibrium demographic processes with marker genetics. The generality of the stage-based approach implemented in this software makes it straightforward to apply to any organism whose life-cycle can be summarized by a Lefkovitch or Leslie matrix.

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KEYWORDS:
seedbank, population genetics, stage-based demography

A metapopulation model for the introgression from Genetically Modified Plants into their wild relatives.

***Patrick G. Meirmans*, Jean Bousquet & Nathalie Isabel**

Most models on introgression from Genetically Modified Organisms have focused on small spatial scales, modelling gene flow from a GMO plantation into a single adjacent population of a wild relative. Few, if any, models have addressed the effect of introgression from multiple plantations into the whole metapopulation of the wild relative. Classical population genetic models can tell us a lot about the fate of alleles in a metapopulation, a system of populations connected by migration; these models may therefore also be applied to study the fate of transgenes that have introgressed into the whole metapopulation. However, the classical models mostly focus on populations that are in equilibrium and they do not include the actual process of introgression over generations, which may be an important evolutionary force in itself. Here, we present a metapopulation model to study the effects of continuous introgression from a GMO into natural populations of a wild relative. One important result of the model is that size of the populations of the wild relative is relatively unimportant. Furthermore, the overall frequency of the transgene in the metapopulation, after a certain number of generations of introgression, depends as much on the rate of migration between natural populations as on the actual rate of introgression from the GMO into the natural populations. This means that studies on the potential ecological risks of GMO introgression should not only look at the rate of introgression and the selection acting on the transgene, but also at the population structure of the wild relative.

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KEYWORDS:
population genetics; selection; introgression; Genetically Modified Organisms; metapopulation.

POSTER

Genetic diversity in anatomical and structural poplar leaf characteristics in relation to biomass production, parentage and canopy position

Najwa Al Afas, Reinhart Ceulemans

The Populus genus is a very rich and variable genus, exhibiting a high variability in morphology, levels of biomass production and resistance to environmental stresses. Early selection of productive and resistance trees based on indirect indicators is an important issue for breeders in order to facilitate the screaming of the most promising genotypes With the objective to highlight the relationships between leaf characteristics versus (i) biomass production, (ii) parentage and (iii) canopy position, anatomical and morphological leaf characteristics were examined for 12 poplar clones belonging to various species, parentages and sections at two canopy positions.

Significant clonal variations in anatomical, stomatal and morphological traits were found. Significant differences were also found between the two canopy positions for both anatomical and morphological leaf traits. Upper canopy leaves had thicker anatomical layers, higher stomatal density, and larger stomatal length than the lower canopy leaves. Leaves of P. deltoides x P. nigra (section Aigeiros) had two palisade parenchyma layers. Leaves of P. trichocarpa parentage (section Tacamahaca) were hypostomatous. Leaf area, specific leaf area, and nitrogen canopy profiles followed the light gradient within the canopy. Above-ground biomass production varied significantly among clones. Significant correlations between biomass production and abaxial stomatal density, as well as nitrogen concentration were found. A principal component analysis (PCA) showed that clones belonging to the same parentage had similar anatomical characteristics, and clones of the same section also showed common leaf characteristics. However, clone Wolterson (P. nigra) differed in anatomical leaf characteristics from other clones belonging to the same section (Aigeiros). Hybrids between the two sections (Aigeiros x Tacamahaca) expressed leaf characteristics intermediate between the sections, while the biomass production was low in these clones.

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Artificial disruptive selection for early height growth in Patagonian Cypress

Alejandro Aparicio[1], **Mario Pastorino y Leonardo Gallo**

Artificial selection against the intermediate phenotypes is not frequent in forest tree species. However, selection of the best and the worst types can significantly contribute to different research lines in advanced stages of a breeding program, such as the formation of a segregating population for the detection of QTLs.

A breeding program has been recently initiated for Austrocedrus chilensis (D. Don) Pic. Ser. et Bizzarri, the Patagonian Cypress, which is the most important productive conifer of the temperate forests in Argentina. Their marginal populations from the steppe have been found to be particularly relevant for breeding as well as for conservation purposes.

In order to analyze differences in the shape of the early height growth curves among progenies and among natural populations of this species, a greenhouse trial with a randomised complete block design was established under controlled conditions of temperature, humidity and fertilization with 158 progenies corresponding to 10 marginal populations from the steppe (N = 3318 seedlings). Height from the cotyledons insertion till the terminal bud of the main axis was measured nine times across the whole second period of growth activity. Based on the final height a disruptive selection was performed at two levels: 1) best 100 and worst 100 individuals of the whole trial, irrespective of their origin or progeny belonging; 2) 21 individuals of each of the best 4 and worst 4 progenies of each origin. A height growth model was adjusted for each group, and differences among the adjusted models for each group at the two levels were tested by means of the Akaike Information Criteria (AICc).

Mean final height of best 100 and worst 100 was 338 ± 45 mm and 126 ± 18 mm respectively (significantly different, $p < 0,0001$; with 512 mm maximum and 65 mm minimum absolute values). In all groups, height growth was best described by second grade polynomial models of the form: $\log_{10}h = a + b.t - c.t^2$ (h = height [mm]; t = time [days]), with significant differences in initial height (a parameter) and growth rate (b and c parameters). An additional observation is useful to illustrate the dispersion of data even within groups: when separating best and worst groups within each group at the level of analysis 2) (progenies selection in each origin), worst group of the best 4 progenies resulted similar to the best one of the worst 4 progenies of the same origin.

Although differences in the initial height account for a great part of the differences among the growth curves of groups at the same level, their differences in the growth rate arise as a character of deeper and more permanent significance, since it describes a continuing process instead of a state.

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KEYWORDS:
adaptive characters; tree breeding; growth models.

Phenotypic differentiation for drought stress tolerance among populations of maritime pine

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Maritime pine (Pinus pinaster Aiton) is an important pioneer species, being used frequently in forestation programmes. The molecular differentiation among populations has been profusely addressed, though not so much information is available on phenotypic traits with a likely adaptive value in response to factors of stress such as drought. Moreover, it has been pointed out a weak correlation between molecular and putative adaptive traits, which limits the use of molecular information in strategies of maritime pine genetic conservation.

In the present work, changes in carbon isotopic discrimination, growth and allometric parameters in response to drought were analysed in four populations of maritime pine covering a latitudinal and rainfall cline across the geographic distribution of the species. Seedlings from four populations were submitted in a greenhouse to a long-term moderate drought.

Water stress brought about a decrease in isotopic discrimination Δ^{13} according to population. Pleucadec (a Landes population) showed a high responsiveness to water stress by decreasing Δ^{13}, whereas Tambrabta (Morocco) had the highest Δ^{13} whichever water stress and no differences were observed between well-watered and drought stressed seedlings. However, Tamrabta increased biomass allocation to roots independently of watering treatment. The differential patterns in Δ^{13} and biomass partitioning among populations pointed out different evolutionary trajectories. It may be hypothesized different strategies of response to drought among populations according to different selective pressures during re-colonization after the last glaciers periods.

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KEYWORDS:

drought, Pinus pinaster, provenances, isotopic discrimination, biomass partitioning.

Molecular genetic analysis of Aleppo pine populations originating from areas of differential forest fire history: Implications for reforestations and breeding

Marina Baka. and *F. A. (Phil) Aravanopoulos*[1]

The molecular genetic diversity of Aleppo pine (Pinus halepensis Mill.), one of the most important Mediterranean conifers with important significant ecological and socio-economic value that has sustained losses due to severe forest fires in recent years, was analysed. There is very limited information regarding genetic marker variation of Aleppo pine forests in Greece, whereas studies linking genetic diversity with forest fire histories are scarce in general. We have sampled a total of 125 trees from five natural populations: Varimbombi (Attica), Istiaea (Euboea Island–Western Aegean Sea), Thermopilae (East Central Greece), Nea Fokaea (Cassandra Peninsula, NE Greece) and Vourvourou (Sithonia Peninsula, NE Greece). These areas are placed geographically on a general southwest (Varimbombi) to northeast (Vourvourou) transect, while at the same time present differential forest fire occurrence and damage indices ranging from extremely high (Varimbombi) to relatively low (Vourvourou). Extraction and isolation of DNA was applied on diploid (embryo) and haploid (megagametophyte) tissue with the use of a modified protocol based on the Dneasy™ Plant Mini Kit (QIAGEN). Eight custom synthesised random decamer primers named Phoenix 1-6 (GIBCO-BRL, Custom Primers) and Phoenix 7-8 (MWG Oligo Synthesis) were employed. In total, 63 loci (7.8 loci per primer on the average) were revealed, while 42 were polymorphic (66.7%). Results indicated high gene flow and absence of inbreeding. When results from embryos and megagametophytes (6 megagametophytes per tree determined maternal genotypes) were compared, the existence of Hardy-Weinberg equilibrium was verified, while no significant differences in genetic parameters were observed. The occurrence of notable molecular genetic variation in Greek populations of P. halepensis was supported, despite the fact that two populations suffered from heavy forest fire losses repeatedly. Populations did not differed significantly among each other in parameters of genetic diversity. High forest fire occurrence and damage (reflected by a forest fire damage index, FDI) was not correlated either positively or negatively with genetic diversity parameters (e.g. gene diversity–H_e, Fig. 1). Geographic and population genetic distances were not significantly correlated (Fig. 2); nevertheless the three groups formed in a respective genetic distance dendrogram (northern populations, southern continental populations, insular population) showed congruence to general geographic locations. The significance of these results in the breeding of P. halepensis and in the planning of reforestation and gene conservation strategies is discussed.

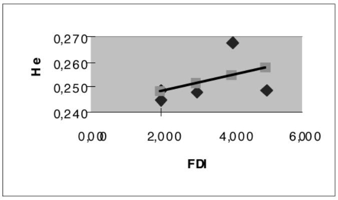

Figure 1

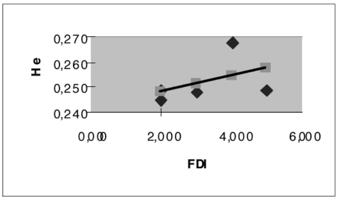

Figure 2

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KEYWORDS:
Pinus halepensis; genetic diversity; molecular markers; forest fires.

I-P5

Ability of Peroxidase patterns in ecotype and phenotype separation of Wild cherry and Mountain ash

Davoud Azadfar[1]

Biochemical markers like as Peroxidase enzymes are very useful in separation of ecotypes, phenotypes or genotypes because of theirs isoenzyme patterns diversity. In this research, twenty individuals of Wild cherry and Mountain ash was signed and sampled for enzymatic studies in two different forests and height from sea level (10 ind. per each elevation). Two years branches of the individuals (5 ind. With Suitable morphology and 5 ind. With unsuitable morphology per each elevation) were collected in the similar ecological conditions and winter. The quantity and quality activities were measured by spectrophotometer in 530 nm and PAGE method respectively during three years continuously. The results indicated that the individuals were separated in four classes for each species. The index isoenzymatic patterns of the individuals with suitable and unsuitable morphology and also two elevations were differed in two or three bands. It was also observed negative correlation between stem quality and quantity activity of Peroxidase. This research indicated valuable genetic diversity of Wild cherry and Mountain ash in Caspian's natural stands. In addition, it was established the role of Peroxidase enzyme as a tree's ecotype and phenotype indicator.

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KEYWORDS:
Peroxidase, Wild cherry, Mountain ash, Ecotype, Phenotype.

Thinning intensity effects analysis on rapid growth of planted Loblolly pine (*Pinus taeda L.*) stands in southern coast of Caspian Sea using statistical MANOVA tests

Amir Bonyad

Loblolly pine (pinus taeda L.) is one of the main exotic species, under commercial plantation in the north of Iran. These plantations are situated in the Caspian forests of Iran, which border the southern coast of Caspian Sea and cover an area of 1.9 million ha. For the present experiment, unthinned, light and moderately thinned stands were selected as three district populations. Data were collected from 180 trees in all, with different stand density. For basal area analysis, 93 plots were measured. Variables such as: diameter at breast height, total height, tree volume, bark thickness, basal area per ha, crown length and crown diameter were measured. Growth rate ratios of dbh in light and moderately thinned stands were 1.09 and 1.18 respectively. MAI of basal area growth rates in two thinned populations were 1.186 and 1.20. Moderate thinning increased the volume of stands by about 20.60% stands volume. This analysis indicated that moderate thinning drastically affects the basal area and the growth rate of volume. After thinning, dimensions of live crowns became greater and more branches became evident. Percentages of live crown ratios in three study populations were calculated as 41.23%, 43.87% and 44.52%. Growth rate interpretations were supported by statistical multivariate analysis of variance using Wilk's (_) and Hotelling's tests. The Wilk's test was carried out on mean vector of 6 variables in three study populations. The null-hypothesis (=14.435>=4.37) was rejected at the 0.01 level of significance. Thus, the results indicated that moderate thinning was significantly influenced the rapid growth rates of planted Loblolly pine stands.

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KEYWORDS:
Caspian Sea, Loblolly pine, multivariate, growth rate, thinning

I-P7

Impact of past fragmentation of the Central African rainforest: phylogeographic studies of Aucoumea klaineana Pierre (Burseraceae).

C. Born[1,2,3], S. Ossari[1], E.J. Wickings[1], M. Hossaert-McKey[2], M.H. Chevallier[23]

The Central African rain forest was very fragmented during globally cold periods occurring at the end of the Pleistocene and between 3500 and 2200 BP, surviving only in a series of relatively small refuges. Paleoenvironmental studies suggest areas that potentially played the role of forest refuges during arid periods for forest species, such as Aucoumea klaineana Pierre (Burseraceae), a forest tree endemic to Central Africa. To understand the impact of past fragmentation of the forest on the distribution of genetic diversity in this species, we determined genotypes for specific microsatellite markers on a large sample (average of 7.91 trees per site for 103 sites) collected from throughout the distribution range of the species. Our results support the existence of at least three forest refuges (Massif du Chaillu, Mont de Cristal, Mont Doudou) in Gabon. Populations in the Congo Republic may have originated from another refuge (perhaps the Mayombe forest), or from the Massif du Chaillu but following an independent colonization pathway. The Mont de Cristal refuge in northern Gabon appears to harbour more genetic diversity than do southern refuges.

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KEYWORDS:

Central Africa, Aucoumea klaineana, phylogeography, forest refuge

Discordant mtDNA and cpDNA phylogenies of the entire genus *Picea* indicate patterns of allopatric speciation related to geographic dispersal and ancient reticulate evolution

Marie Bouillé **and Jean Bousquet**

In the Pinaceae, cpDNA is paternally inherited and mtDNA is maternally transmitted. Such a contrast offers an opportunity to study the evolutionary history of Pinaceae genera from different perspectives, one from dispersal of paternal lineages through pollen and seeds and one from dispersal of maternal lineages through seeds only. We have determined the phylogeny of the entire genus Picea by sequencing three cpDNA regions (trnK, rbcL, and trnTLF) and the intron 2 of the mitochondrial gene nad1 for 35 taxa. Intraspecific sequence variation was minimal as compared to interspecific divergence. Phylogeny was estimated using parsimony and bayesian approaches. Partition homogeneity tests indicated that the strict consensus derived from the chloroplast trnK was significantly different than that obtained using the chloroplast rbcL and trnTLF regions. Each of these as well as that obtained by considering the three cpDNA regions was different than the mtDNA strict consensus. None of the phylogenies matched current taxonomical classifications either based on reproductive structures or vegetative characters. Differences between cpDNA partitions suggested heterologous recombination linked to ancient reticulate evolution. The mtDNA phylogeny obtained was geographically more structured than either of the cpDNA phylogenies. For instance, most North American taxa formed a monophyletic group on the mtDNA strict consensus, and they were regionally well structured, indicating allopatric speciation by dispersal and isolation. Similar patterns were found among Asian taxa. Most taxa at the base of the mtDNA tree were from Asia, indicating this region as the putative center of origin for the genus. Incongruences between cpDNA and mtDNA phylogenies pointed at ancient lateral transfers of the chloroplast genome between mtDNA lineages. Such reticulate evolution is in agreement with the weak reproductive isolation and high crossability observed today between many taxa. The results indicate the reduced value of cpDNA as a phylogenetic and phylogeographical marker in genera with similar paternal transmission of the chloroplast genome.

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KEYWORDS:
cpDNA, mtDNA, phylogeny, phylogeography, Picea, spruces.

Annual variation of pollen-mediated gene flow in *Quercus petraea*

***Jutta Buschbom*, Armin König, Bernd Degen**

Pollen-mediated gene flow is one of the most important population-genetic processes in wind-pollinated tree species. Pollen availability and dispersal in wind-pollinated species is shaped by the distribution of reproductive trees, the flowering phenology and meteorological conditions during the flowering period. The quantity and patterns of successful gene flow events determine the population-genetic characteristics of the next generation, as for example outcrossing rates and hybridization events between closely related species.

The yearly variation of gene flow within a mixed oak stand consisting of Quercus petraea and Q. robur in northern Germany was investigated using parentage analyses and a two-generation approach. The characteristics of the pollen cloud and the effective pollination neighborhood as well as the extent of hybridization were investigated and compared for two years. The study is based on the genetic analysis of 20 acorns of each of 20 Q. petraea trees and the genotyping of 500 potential pollen donors of both species. Unambiguous characterization of adult trees and progenies is ensured by genotyping all individuals at six nuclear microsatellite loci. The study provides insight into the variability of pollen-mediated gene flow in wind-pollinated trees.

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Ecophysiological and genetic differentiation of American live oaks (*Quercus* section Virentes) from the tropics to the temperate zone

***Jeannine Cavender-Bares*[1], Annette Pahlich[1], Nicholas Deacon[1] and Jessica Savage[1]**

An unresolved question in ecology is the extent to which broadly distributed species are comprised of a small number of genotypes with broad physiological tolerances or of many locally adapted genotypes. The live oaks (Quercus section Virentes Nixon) form a species complex that occurs in lowland and coastal forests from the dry tropics of Costa Rica to temperate North Carolina. Two broadly distributed and introgressing species, Q. virginiana and Q. oleoides, together span the entire range and were the focus of this study. Based on neutral chloroplast and nuclear markers, we used a Bayesian clustering approach and conventional population genetic approaches to determine the underlying genetic structure across 10 geographic regions. We sought to determine whether genetic structure corresponded to geographic distance and/or to the species designations of the two species. Chloroplast sequence data revealed two broadly distributed ancient haplotypes that span both species with several more recent haplotypes derived from these. Nuclear microsatellites revealed population structure that parallels, in part, species boundaries. Surprisingly, both data sets supported a disjunct Costa Rican population that does not correspond to an existing species boundary.

In a separate study, we tested whether ecophysiological traits of four populations showed differentiation corresponding either to neutral genetic variation or to variation in native climate. In a greenhouse experiment, live oak seeds from Costa Rica, Belize, northern Florida and North Carolina were grown under contrasting temperature and drought regimes representing the extremes of the live oak range. Ecophysiological traits related to cold tolerance clearly corresponded to climate and latitude. After exposure to cold temperatures, northern populations were able to prevent freezing damage but southern populations were not. Northern populations also showed greater leaf shedding in response to cold temperatures and lower freezing-induced xylem embolism. In contrast, differences among populations in drought tolerance were less clear, despite large differences in rainfall patterns. Other morphological traits, including leaf size, paralleled taxonomic boundaries, while many traits showed no population-level differentiation. These studies show that individual traits vary independently among populations, and do not necessarily correspond to variation in genetic markers.

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KEYWORDS:
population structure; microsatellites; chloroplast markers; freezing and drought tolerance.

Marginal populations as a potentially valuable genetic resource in Spanish black pine (*Pinus nigra* subsp. *salzmannii*).

***José Climent*[1]*, Santiago C. González-Martínez[1], Álvaro Soto[2], Marta Pardos[1], Ricardo Alía[1].**

Marginal populations of forest trees are often the object of contradictory interpretations regarding their actual role in the evolution of the species or their relevance for genetic conservation. In recent years, despite an abundant literature focused on population dynamics and population genetics of marginal populations, still few data are available on their potentiality for deployment in forest management or conservation programs. The Spanish black pine (Pinus nigra subsp. salzmannii) is a good example. The Iberian distribution of this species consists of a mixture of continuous forests and isolated stands, ranging from several hectares to a few individuals. These isolated manifestations tend to occur on sub-optimal or extreme environments, showing often decline and low recruitment due to the on-going climate change. Our aim in this study was (i) to determine whether seedlings from the marginal populations are well adapted to difficult environments and (ii) to compare genetic variability among central and marginal populations of Spanish black pine using both molecular markers (chloroplast microsatellites) and quantitative traits. Fourteen native populations of black pine covering most of its natural range, including marginal and central populations, were selected for this study. We carried out a common garden experiment in a cool greenhouse, with two watering regimes plus a drought-survival treatment using a continuous decrease of water availability. Germination, ontogeny, height growth and survival were periodically measured, and biomass components and leaf area were obtained in two harvests. Finally, six cpSSRs were scored in 24 unrelated trees from each population. Marginal populations showed lower growth, not fully explained by their slower germination. Survival of the southern marginal populations was generally higher, associated to a higher root mass fraction, higher relative growth rates in the low-watering treatment and delayed ontogeny. By contrast, northern marginal populations behave more like central populations. Levels of molecular diversity in black pine were high, although a notable reduced diversity is apparent in some of the southern marginal populations. At the light of our results, both main geographical (north-south) trends and the degree of marginality seem relevant in the behaviour of Pinus nigra salzmannii populations. Considering both seedling performance and neutral molecular markers most of the marginal populations assessed seem appropriate for their deployment in restoration forestry if the target is self-maintaining and gene conservation rather than fast growth.

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KEYWORDS:
marginal populations, drought adaptation, phenotypic evaluation, chloroplast microsatelites.

Genetic variation of *Pinus pinaster* to cold and water stress

***Leyre Corcuera*, Eduardo Notivol, Eustaquio Gil-Pelegrin**

As part of a program to evaluate the genetic resources of maritime pine (Pinus pinaster Ait.) for drought and cold tolerance, we examined several physiological traits from three seed sources in two hierarchical provenance-progeny trials. Variation in stability of photosynthetic apparatus, by chlorophyll fluorescence and gas exchange measurements, and leaf-level water use efficiency, estimated by carbon isotope composition, were assessed during late winter of 2006. The aims of the study were (i) to estimate the intra and inter population variation in physiological parameters related to drought and cold tolerance and (ii) to evaluate the relationship between physiological traits and the geographic variation of climatic conditions of the trials and seed sources.

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KEYWORDS:
genetic variation, populations, intrinsic water use efficiency, chlorophyll fluorescence, gas exchange

Variation in phenotypic plasticity of early growth in a *Pinus pinaster* Ait. Atlantic provenance

***Raúl de la Mata*, Rafael Zas and Esther Merlo**

Two different environmental regions can be clearly differentiated in Galicia (NW Spain), the coastal and the interior area. Both regions differ mainly in the summer drought intensity and the annual temperature oscillation. Although Pinus pinaster lives and is planted in both regions, several reasons (unknown genetic origin, lack of adaptation, poor quality stands, …) impede the establishment of a classic breeding program in the interior area. As an alternative, the analysis of the plasticity to drought and temperature oscillation of the genetically improved coastal material is highly desirable. This information can be used to explore the viability of using the coastal material in the interior area and also, to improve our knowledge about how the breeding material will respond under the predicted climate change. Previous research under controlled conditions suggested an important genetic variation in phenotypic plasticity to drought within the coastal breeding population. Here, we present the analysis of the variation in phenotypic plasticity of the total breeding coastal population in field conditions. Open pollinated families of 116 superior trees included in several seed orchards in the coastal region were planted in 7 locations, covering a wide range of environmental conditions, from clearly Atlantic climate to interior areas with intense summer drought and high annual temperature oscillation. Height increment (total height – nursery height) and survival one year after plantation was measured and used for the analyses. Differences in plasticity were analyzed using the joint regression analysis, the analysis of genetic correlations between sites, and using the additive main effect and multiplicative interaction (AMMI) model. The results show markedly intraspecific variation in phenotypic plasticity with different adaptation capacities to the environmental gradient among the studied families. The results are discussed in terms of global climate change and in relation to exploit these differences towards selecting the coastal material suitable for reforestation and/or breeding in the interior area.

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KEYWORDS:
genotype x environment interaction, maritime pine, stability, tree breeding, half-sibs, progeny trials

Seed and cone traits in a polycrossing hybrids populations of maritime pine. Indicators of hybridization viability

***Raúl de la Mata*, Esther Merlo and Rafael Zas**

Within the frame of the Galician (NW Spain) Pinus pinaster breeding program, inter-population hybrids were generated to combine desirable characters, and to provide new material better adapted to extreme environmental conditions. Two sets of polycrossing factorial mating designs were implemented in 2003 in two clonal seed orchards. Eight selected female parents of the first orchard (a first generation orchard of the Galician-coastal breeding population) were pollinated with seven pollen mixtures of the Galician-interior, Corcega, Boniches (Cuenca), and Garganta (Landas) provenances. In the second orchard (a first generation orchard of the Soria-Burgos provenance), five female parents were pollinated with four pollen mixtures of the Galician-interior provenance and of the Galician-coastal breeding population. In this study we examine cone and seed morphometric traits in order to know the variation of reproductive characteristics among and within the hybrid populations, and to provide indicators of possible reproductive barriers. Ten hybrid populations, including 31 and 20 hybrid families in each orchard, and two open pollinated populations with eight and five families in each orchard, were compared. A total of 298 cones were analyzed. Cone weight, length and width, the number of scales and the number of aborted, empty and filled seeds per cone, and the weight of the filled seeds were measured. The ratio of the weight of filled seeds to the cone weight was considered as the reproductive efficiency, whereas the proportion of empty seeds with respect to the total developed seeds as an index of allelic incompatibility. At the population level, significant differences were observed for all traits except for the mean weight of the filled seeds and the number of scales per cone. At the family level, the paternal pollen mixture significantly influenced the number of aborted or non-pollinated ovules and the number of filled seeds per cone in the two factorial sets. The maternal parental effect was significant for the cone weight, the filled seed weight, and the number of filled seeds per cone. The highest reproductive efficiency was obtained for the open pollinated cones. We discuss the utility of the results for determining the heritability pattern of the studied traits, the hybridization viability, and to explore the suitability of these traits for differentiating populations.

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KEYWORDS:
hybridization viability, reproductive efficiency, seed and cone traits, index of inbreeding, Pinus pinaster

The origin of middle Polish disjunction in Norway spruce range: a phylogeographic approach.

Andrzej Lewandowski, ***Monika Dering***

We applied mitochondrial DNA marker to provide evidences about the following issues: (i) contribution of Norway spruce (Picea abies (L.) Karst.) glacial refugia in the postglacial re-colonization of Poland; (ii) the postglacial migration pathways of Norway spruce in Poland; (iii) origin of middle Polish disjunction in the European range of Norway spruce. To unravel the postglacial history of Norway spruce, genetic variation at mitochondrial nad 1 b/c via PCR-RFLP was assessed in 29 populations distributed across the north-eastern and southern part of natural range of Norway spruce in Poland. The two mtDNA haplotypes identified point out the Russian and southern refugia as an origin in postglacial re-colonization. Obtained results clearly demonstrate that the central Russian refugium has formed the northeastern range of Norway spruce in Poland. The genetic data do not allow Carpathian and Alpine refugia to be distinguished. However, if we take the palynological data into account, the most probable scenario would be the colonization of southern part of Poland from Carpathian refugium. Genetic data suggests also that the middle Polish disjunction in Norway spruce European range is probably of anthropogenic origin as the contact zone between the two migration paths was delineated.

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KEYWORDS:
Norway spruce; postglacial re-colonization; nad 1 b/c; PCR-RFLP; middle Polish disjunction

Variation of some phenological and pathological traits among several *Prunus avium* Spanish populations

Raquel Díaz **and Josefa Fernández**

Wild cherry (Prunus avium) is one of the most valued Spanish forest tree species and is considered a noble hardwood. However, little information is available about its intraspecific variation and adaptability in this country. Present work studied the variation of two phenological traits (bud burst and flowering) and one pathological trait (leaf spot) of ten populations, covering the Northern part of the Spanish distribution area. Data were assessed during year 2005 in a clonal seed orchard located in Tui (Pontevedra, Spain). Experimental design was a randomized complete block design, with 10 populations, 129 clones (9 to 19 clones per population), 10 blocks and a one-tree plot. Variation within populations was higher than variation among populations, even though both of them were significant in all cases. Broad-sense heritability was very high for all traits ($h_C^2>0.80$). All the studied traits were highly correlated. The populations whose buds burst the earliest had an earlier flowering. Furthermore, the greater the susceptibility to cherry leaf spot, the earlier the populations. Correlation analyses between the studied traits and geoclimatic factors indicated the existence of clinal variation, responding to altitude and temperature of the hottest month.

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KEYWORDS:
Wild cherry, leaf spot, bud burst, flowering, geographic variation, genetic variation.

Wild cherry reproductive phenology

Pablo Castelo, Esther Merlo, *Raquel Díaz* and Josefa Fernández.

The knowledge of the reproductive phenology of a species is essential both, in a breeding and a conservation program. Flowering determines reproductive success and thence is a really important trait to understand natural selection, population diversity, gene flow or genetic isolation.

As far as we know, there is no knowledge about the relationship between the different de visu morphological flower stages and the stigma receptivity and pollen emission. The most advanced phenological stage in a tree is the easiest data to register when assessing flower developmental data. For that reason, present work characterizes:

Stigma receptivity and pollen emission under defined visual phenological stages to clarify the relationship between stigma receptivity and pollen emission in one hand and different developmental stages in the other.

Relationship between the most advanced phenological stage in a tree and the flower percentages in the reproductive stages (i.e. when the stigma is receptive and/or the pollen emission has started).

Nine flower developmental stages were considered when assessing data in a Prunus avium breeding population from northern Spain, ranging from closed buds to flowers in which petals were all abscised. An electronic magnifying glass was employed to examine stigma and pollen development at the four stages of the flower development ranging from flowers in which petals were starting to open to flowers in which petals were all abscised. Three out of the four stages were made up related to different degree of stigma receptivity and pollen emission. Flowers in the last considered stage (i.e. flowers in which the petals were all abscised) had no pollen emission or stigma receptivity at all.

From the relationship established between the most advanced flower developmental stage and the flower percentages in the fertile stages, we want to outlined that the highest percentage of fertile flowers was obtained when the most advanced stage correspond to flowers in which petals were abscising. As an example, the reproductive phenology of a P. avium clonal seed orchard is presented.

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KEYWORDS:
Prunus avium, flowering, stigma receptivity, pollen emission.

I-P18

Diversity and genetic structure of *Pinus uncinata* at chloroplast microsatellite loci

Artur Dzialuk[1] ; Ewelina Muchewicz[2] ; Adam Boraty_ski[2]

The mountain pine (Pinus uncinata) occurs in the Pyrenees and the western and central Alps, the Central Massif, Jura, Voges and the Ligurian Appenines. It forms forests at the elevations of 850-2700 m above sea level. The systematic position and genetic diversity of the species are still not sufficiently known.
In this study, ten chloroplast, paternally inherited simple-sequence repeat markers were used to describe genetic variation in eleven populations covering the entire range of the species.

The 55 size variants defined 124 different haplotypes among 319 individuals analyzed. The majority of haplotypes (60%) were detected only once. Our results indicate very high level of within-population haplotypic diversity (H_e = 0.980). The AMOVA showed low (but significant) differentiation among populations (_$_{ST}$ = 0.05, P = 0.010). The average mean distance among individuals within populations, according to an SMM (), was 4.86.

The genetic structure observed in P. uncinata is very similar to those reported for the Iberian populations of P. sylvestris. The implications of these results for the taxonomy of the mountain pine are discussed.

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KEYWORDS:

biodiversity; gene diversity; population differentatiation; cpSSR; Pinus uncinata.

Minimisation of inbreeding: a new algorithm for the establishment of seed orchards or conservation plots

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The increase in the level of inbreeding in a population comes together with deleterious effects on different productive traits and on the fitness itself. Therefore, it should be avoided. At the same time, in conservation populations or those intended to the reinforcement of endangered populations, it is also crucial the maintenance of high levels of genetic diversity. Inbreeding arises from low population census sizes and/or the mating between genetically related individuals. Consequently, one of the proposed management strategies is to avoid as much as possible the mating between relatives. In open-pollinated tree plantations the usual way to avoid inbreeding is to separate relatives (i.e. to plant them far away from each other), as the probability of pollination between two individuals is inversely proportional to distance, usually following an exponential dispersal function. Traditionally, the only information about relatedness was the clone to where the tree belonged and, thus, design methods for seed orchards were based on avoiding two individuals from the same clone planted together and in allocating the highest number of different clones in the surroundings of a mother tree (e.g., neighbourhood permutation schemes). The increasingly availability of molecular markers allows for the estimation of coancestries between trees. In the present study, we have developed an algorithm based on mathematical programming to determine the optimal allocation of trees for minimising the probability of generating inbred offspring. Several are the advantages of the method: i) it is able to include all available information about the relationships between trees; ii) it accounts for the global probability of generating inbreeding and not only the influence of neighbours; and iii) it is flexible about the assumed pollen dispersal function, allowing the adaptation to the particularities of the reproduction of each species.

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KEYWORDS:
tree allocation design, inbreeding, pollen dispersal kernels, relatedness estimation

I-P20

Population structure of wild and domesticated chestnut (*Castanea sativa* Miller) using isozymes and quantitative traits

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Most area covered by mono-specific chestnut (Castanea sativa) forests in Spain are orchards and coppices cultivated for the production of nuts and wood. There are evidences of both types of management in western Europe since Middle Ages. The genetic impact of orchards in the wild gene pool depends on their monoclonal or polyclonal structure, the adaptive characteristics of varieties and the antiquity of cultivation. The frozen genetic structure of old coppices through successive rotations, has avoid natural selection and this diminished the possibility of increase adaptation to local environment. During last decades with the abandonment of agriculture regeneration from reduced stocks is abundant in mixture with other species in recolonization events. A research was initiated with the following objectives : i) to describe the geographic structure of wild populations; ii) to determine the origin of varieties grafted in orchards; iii) to describe the within populations diversity. Six populations were selected through the Spanish chestnut area, each one composed by two subpopulations, one of grafted trees and the other of wild trees. The study was performed on basis of isozyme analysis of buds collected in situ, nut ripening dates recorded in situ and three adaptive traits (height, bud set and flushing) recorded in a field trial experiment, which was planted with open pollinated progenies collected in individual trees within each subpopulation. Wild subpopulations had minimum values of diversity (He, P, Ne) in the extreme southern population in Gaucín (Málaga) and the maximum values of effective population size were in two coppices in Central East Spain (Guadalupe) and As Médulas (León). The multilocus genotypic differentiation was high in all wild subpopulations ($\delta_{T(ml)}$ =1-0.97). Two types of orchards were identified: monoclonal orchards ($\delta_{T(ml)}$=0) and polyclonal orchards ($\delta_{T(ml)}$ =0.31-0.86). Wild populations from dry areas flushed and formed the bud earlier than populations from humid areas. The correlation between budset and flushing and drought index has indicated directional selection as the cause of geographic structure of wild populations. Patterns of variation of orchards were similar to the wild ones in the same site except for Guadalupe and As Médulas, which seem to be introduced from humid areas. The adaptive potential of subpopulations was estimated with the genetic coefficient of variation for three adaptive traits. It seemed to be enough to counteract climate change in wild subpopulations but null for orchard ones. Genetic parameters estimated from isozymes have indicated founder effects in some wild populations originated from polyclonal orchards. Local wild chestnut populations not affected by recent founder effects are the gene resource to be considered for biological conservation. They would be taken into account as well as for the selection of production populations for wood production due to its diversity and adaptation to local conditions. Old grafted varieties are the gene resource to be considered for the production of nuts and in some specific cases for wood. Grafted orchards, especially the monoclonal ones, must be considered a threat to the genetic variability of local wild gene resources.

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KEYWORDS:
origin of grafted varieties, flushing, budset, adaptive potential, additive coefficient of variation.

Ice Age III – or where have all the poplars gone?

Barbara Fussi[1] **and Berthold Heinze**[1]

Vienna and the Danube River are host to two ecologically important tree species of the genus Populus: the European Aspen (Populus tremula) ant the white poplar (Populus alba). Both have extended their natural ranges to this area after the last glaciation. Lacking pollen data for poplar hampers the search for glacial refugia – whereas latter is assumed to be found in Southeastern Europe. The objectives of this study are to find out about the post-glacial migration paths of both species by tracing their chloroplast DNA.We compare plant samples collected in the Central and Southeastern European region (Austria, Cech Republic, Slovakia, Hungary, Croatia, Romania, Bulgaria) by applying PCR-RFLP on chloroplast DNA.

Moreover the two species hybridize quite frequently (Populus x canescens) what makes it difficult to identify potential study material. We were successful in developing 8 cpDNA restriction site polymorphisms dedecting mutations in both parental species for clear separation. The eight haplotypes were mostly found within P.alba-like plants – only one haplotype was typical of P.tremula.

The work is still in progress and results will be presented.

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KEYWORDS:
Populus, cpDNA, PCR-RFLP, phylogeography, post-glacial recolonisation

Analysis of genetic variation in Indonesian *Shorea* (Dipterocarpaceae) species: AFLP and SCAR markers

***Oliver Gailing*, Cui-Ping Cao, Hani Nuroniah, Yanti Rachmayanti, Iskandar Siregar*, Ulfa Siregar*, Reiner Finkeldey**

Many tropical forests in Southeast Asia are dominated by dipterocarps with Shorea as the largest genus of the family. They are the main sources of the world´s demand for plywood made from hardwood. Recently, due to the exploitation for their valuable timber, Shorea forests are heavily affected by deforestation and forest fragmentation. We analysed (1) the genetic variation within and among geographically distinct populations of the most common and widespread emergents of lowland dipterocarp forests in Indonesia, Shorea leprosula and Shorea parvifolia, and (2) the variation among nine Shorea species at two locations on Borneo and Sumatra, respectively. A pronounced genetic structure was detected within and among species. Combinations of AFLP (Amplified Fragment Length Polymorphism) markers could be extracted that are diagnostic for geographic regions in S. leprosula and S. parvifolia. Other AFLP markers distinguished different Shorea species from each other. Diagnostic AFLP fragments have been extracted from polyacrylamid gels and their DNA sequence was determined. The objective is to develop a set of PCR markers (SCARs = Sequence Characterized Amplified Regions) from AFLPs and other informative markers (cpDNA) that allow to distinguish among geographic regions of important Shorea species and that can be used in combination with other markers to differentiate between species. A practical application is the development of specific PCR primers in order to amplify very short polymorphic regions in degraded DNA probes from wood samples. These markers will be used to identify wood from different geographic regions of Shorea species within the context of forest certification.

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KEYWORDS:
dipterocarps, Shorea, AFLPs, marker development, wood certification

Proteomic study of wood in formation in maritime pine

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The wood is a highly variable material at the chemical and anatomical levels, as in its mechanical properties. The compression of the molecular mechanisms implied in the wood formation, pass by the study of the genome expressed during the xilogenesis, at a transcriptomic and proteomic levels.

Along with the genetic variation, the environment and the age of the cambium also affect the properties of the wood. In this study, we test the hypothesis that this variability can be bound to the differential expression of proteins during the formation of the wood.
In one first stage, we carry out the analysis and comparison of the protein maps obtained by bidimensional electroforesis of total proteins extracts of xilema in differentiation according to

a cambial age gradient (4 levels in 4 repetitions, from mature wood in the base of the trunk to juvenile wood in the top of the tree). After image analysis and statistical analyses, those proteins that displays differential accumulation were characterized systematically by spectrometry of masses in tandem (ESI MS/MS). In parallel, the same samples were anatomically and chemically characterized.
In the 2003 season, a total of 1,372 spots were placed on the reference map. This reference map was used to compare the abundance of each spot/protein (spot volume) between the 4 levels. ANOVA was used to select spots/proteins that showed significant differences between the four levels. Altogether, 231 spots showed significant variations between the four levels analysed of the cambial age gradient. A total of 34 spots were selected to be analysed by LC ESI MS/MS, 22 were over-expressed on mature wood and 11 on juvenile wood.
In the 2005 season, a total of 743 spots were placed on the reference map. The spots volume dataset was analysed by ANOVA. This led to the selection of 267 spots that showed a significant difference ($p<0.001$) between the levels of cambial age. A total of 44 spots were selected to be analysed by LC ESI MS/MS, 33 were over-expressed on mature wood and 11 on juvenile wood.

Based on the functional categories of the oveexpressed proteins and their fold change, in the mature wood are overexpressed proteins involved in defense and stress response and in juvenile wood proteins related to cellular division and genes and protein expresion.

The cellular functions of the proteins identified are consequent with the results obtained in chemical and anatomical analysis. The use of complementary analisis like proteomics, transcriptomic, chemical and anatomical analysis in wood formation, are helping giving new clues in try to understand the complex process of wood formation.

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Clinal patterns of nucleotide diversity at candidate genes for drought stress tolerance in a maritime pine metapopulation: how can we detect them?

***P. Garnier-Géré*[1], V. El Mujtar[2], V. Léger[1], E. Eveno[1], P.Léger[1], C. Plomion[1]**

In the context of a trinational project that aims at identifying genes and mutations of adaptive significance, clinal patterns of nucleotide diversity and differentiation are analysed in Pinus pinaster natural populations, for candidate genes of drought stress tolerance. Five populations have been sampled along a rainfall gradient in central Spain, and 10 fragments (>7 kb) have been retained a priori for their high polymorphism and sequenced in at least 10 gametes per population. These fragments belong to 6 candidate genes involved in cell wall formation and general drought stress response. A ten-fold range of variation of nucleotide diversity estimates was observed among gene-fragments (from around 0,001 to 0,015 for π_m per site), which is comparable to published estimates of diversity in selected fragments of candidate genes for adaptive traits. Very little nucleotide differentiation was detected in this set of genes, which raises the issue of diversifying selection efficiency (and its detection at the molecular level), when a high gene flow through pollen occurs along the cline as it is the case in this species. Most genes however showed significant departures from neutrality, which are most likely due to selective rather than demographic effects. Strong haplotype structure in most genes, and Tajima's D or Fu's Fs statistics significantly different from zero (neutral case) are consistent with either simple or more complex signals involving balancing or frequency-dependent selection. Nucleotide diversity and selection signatures will be compared between populations along the cline.

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KEYWORDS:
adaptation; drought stress; nucleotide diversity; Pinus pinaster; balancing selection

The impact of landscape disturbance on fine-scale genetic structure in the Guanacaste tree (*Enterolobium cyclocarpum*) in Costa Rica.

***E Gonzales*[1], JL Hamrick[2], PE Smouse[1] and R Peakall[3]**

We have analyzed fine-scale genetic structure in the Guanacaste tree, Enterolobium cyclocarpum, as influenced by landscape disturbance in Guanacaste Province, Costa Rica. We have sampled four sites (a relatively undisturbed national park, two cattle ranches, and a small pueblo). Using 13 allozyme markers and a series of distance classes extending out to four km, we have used novel statistical methods to conduct a multi-locus spatial autocorrelation analysis for each site separately and for the pooled array. Spatial autocorrelation is detectable out to at least 500 m, except in the pueblo. Within the continuous tropical dry forest of Palo Verde National Park, autocorrelation is quite strong at distances < 250m. Within heavily cleared ranch sites (Hacienda Solimar & Rancho Stewart), short-distance autocorrelations are smaller but significant. For the trees in urban environment with a longer history of disturbance (Pueblo Bagaces), there is no evidence of autocorrelation at all. The results suggest that the naturally established within-population genetic structure of this species in native forest settings decays progressively, under the influences of human disturbance over a few decades. While there seems to be no diminution of genetic variation, the fine-scale structure within populations dissipates with continuing anthropogenic disturbance.

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Isolation and the antifungal activity of defensin-like protein from Scots pine roots

Valentina A. Kovaleva[1], ***Roman T. Gout***[1]

Plant-pathogen interactions have been studied extensively in horticultural crops but relatively little work has been done on tree pathosystems. Crop studies have led to the selection of disease resistant varieties and have improved our understanding of gene regulation in response to pathogen. It is well known that pathogenic infection of conifers causes annual economic losses of billions Euros in EU countries. To protect themselves against pathogen attack, plants produce a wide range of antimicrobial compounds, such as defensins. Seed germination is likely to be one of the most vulnerable periods for pathogen attack in a plant's life cycle. The recent data have indicated that Norway spruce produces defensin to prevent pathogen infection at this stage of the life cycle.

In this work we describe the first successful isolation of the endogenous defensin from gymnosperm. Using affinity purification approach on phosphotyrosine column, we have isolated protein from seedling roots of Pinus sylvestris and identified it by mass spectrometry. This protein was found to be defensin-like protein.

The Scots pine defensin was purified by acid extraction, ammonium sulfate precipitation and the ion exchange chromatography on phosphocellulose. It exhibited a molecular mass of 9,8 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This protein was active at concentration < 10 μM against phytopathogenic fungi Fusarium oxysporum, Botrytis cinerea, Pythium debapyanum. Fungal inhibition occurred with hyphal branching.

Extensive bioinformatic analysis indicated that cDNAs corresponding to the Scots pine defensin are not present in public databases (there are EST clones corresponding to other conifers). To determine the full nucleotide sequence of the Scots pine defensin, we have recently initiated the production of a cDNA library from the roots of 7 days old seedlings.

Based on our findings, we suggest that the Scots pine defensin is promising as a candidate to be included in a molecular breeding program for Pinus sylvestris aimed at increasing of productivity and biological resistance of forest ecosystems, specifically relating to the protection of conifers from phytopathogenic agents.

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KEYWORDS:
defense response; plant defensin; Pinus sylvestris; isolation

A Gibbs sampling method for estimation of additive and dominance quantitative genetic parameters

Patrik Waldmann[1], Fabian Hoti[2], *Jon Hallander*[1] and Mikko J. Sillanpää[2]

In forest tree breeding programs almost all attention has been paid to the additive genetic component. Recently, several publications have shown that non-additive genetic variance can be converted into additive variance in after population bottlenecks, founder events and directed truncation selection. This phenomenon has been proven both theoretically and experimentally using different model organisms. An increase in additive variance will result in an increase in heritability which is very important since it determines the possible selection advances for a certain character in a breeding population.

The individual tree model combines fixed and random (genetic) effects to a phenotypic value for each individual in a population and utilize the relationship between all individuals. This makes the individual model a very powerful tool to estimate genetic variance components and individual breeding values, which can be used to rank the selection indices of individuals. Also, pedigree structures can have a general and flexible design, and do not need to be static (e.g. only full-sib families). The individual model can easily be extended to incorporate dominance genetic effects.

The recent improvements in computational power have increased the possibilities to use computer intensive statistical methods in quantitative genetics. Such statistical inference methods are Bayesian Markov chain Monte Carlo (MCMC) methods, which constructs a Markov chain to get samples from its stationary distribution. The Gibbs sampler is a MCMC method that successively samples from conditional distributions of all parameters in a model in order to generate a random sample of the posterior distribution, which is the target for Bayesian inference. The Gibbs sampler has frequently been used in animal breeding, but rarely in tree breeding.

Here we present a new Bayesian Gibbs sampling approach for estimation of additive and dominance genetic variances using the individual infinite allele model. The performance of the method is illustrated using real data from a partial diallel study on Scots pine (Pinus sylvestris) from northern Sweden.

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KEYWORDS:
quantitative genetics, dominance, Gibbs sampling, Scots pine

Landscape genetics of initial recruitment within and around a mixed oak forest fragment

***Arndt Hampe*, Begoña Garrido and Rémy J. Petit**

In many regions across Europe, large-scale afforestation with conifers has resulted in the reduction of autochtonous broadleaved forests to small, fragmented stands, whose patterns and dynamics of regeneration remain little investigated. This study explores the initial recruitment, that is, the arrival and establishment of propagules, within and around a mixed oak forest fragment (including Quercus robur and Q. pyrenaica) surrounded by pine plantations. Over a total area of 6 ha, we mapped, marked, and monitored all seedlings emerging away from adult oak canopies, as well as 20% of the seedlings found beneath adult oaks (total: 865 individuals). Their growth habitat was recorded at three spatial scales, and tissue samples were taken for molecular analyses. Likewise, all adult oak trees of the area were mapped and sampled.

The spatial distribution of seedlings was highly heterogeneous. Besides an expectable peak density beneath adult oaks, we also found remarkably high densities in several areas with low or absent vegetation, as well as some individuals emerging in the understorey of pine plantations. In contrast, large areas dominated by bracken (Pteridium aquilinum) were almost completely devoid of seedlings. Both the location and the microhabitat of seedlings observed away from oak canopies suggest that most of them have likely been dispersed by jays (Garrulus glandarius), although some acorn dispersal by rodents cannot be excluded. Seedling survival varied significantly across the study area.
At present, microsatellite analyses are conducted in order to: 1) describe the spatial genetic structure of seedlings across the heterogeneous vegetation mosaic, 2) identify the parent trees of seedlings and reconstruct the population's seed and pollen dispersal kernels, and 3) test if a relationship exists between the genetic makeup and the survival rate of seedlings.

Results should help to better understand the ecological and population genetic dynamics of oak regeneration under field conditions and to provide practical guidelines for forest management, e.g. during reconversion of conifer plantations into broadleaved forests.

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KEYWORDS:
acorn dispersal; microhabitat; Quercus robur; Quercus pyrenaica; seedling survival

Population genetic processes in *Abies* seed orchards

Ole Kim Hansen[1], Erik Dahl Kjær[1] & Ulrik Bräuner Nielsen[1]

Abies is a widespread genus in the northern hemisphere, and the approximately 40 species deliver a wide range of ecological as well as commercial utilities. Several of the Abies species are used for production of Christmas trees and greenery in Denmark, and for two of the species (Abies nordmanniana and Abies procera) breeding programmes are currently running. Employment of clonal seed orchards (CSOs) is the backbone of these programmes, and the population genetic processes in the orchards are crucial in the optimization of this operational phase of the breeding. Dysfunctions such as asymmetric contribution of pollen, selfing, pollen contamination, hybridisation and grafting/labelling errors may all hinder that the potential genetic gains are transmitted to the forestry sector, and thereby utilized in real-life.

We use microsatellites to investigate different aspects of CSO dysfunctions as well as barriers to hybridization. This is done by paternity analysis in open-pollinated seed lots, as well as in seed lots produced by controlled crossings using pollen-mixes.

The poster shows results and ongoing activities from different experiments. The expected implication of asymmetric contribution of pollen on the genetic value of the seed from the CSOs is also estimated.

Studies of population genetic processes in CSOs do not only have practical/commercial value, but are also a convenient way of studying processes related to evolutionary dynamics and genetic conservation, e.g. through hybridisation studies, studies of selfing, gene-flow assessments etc.

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KEYWORDS:
Abies; population genetic processes; seed orchard dysfunctions; paternity analysis.

I-P30

Genetic structure of natural and planted populations of *Fagus crenata* in Miyagi prefecture, Japan

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Reforestation with nonlocal seed sources may result in genetic disturbance and maladaptation to local environments. Therefore, it is important to use a local seed source within a seed zone delineated by the phylogeography of the species. This study can be regarded as a model for such a local seed zone for Fagus crenata, a dominant tree species in the cool temperate region of Japan. Chloroplast DNA and nuclear microsatellite variations were investigated in natural populations of the species in Miyagi prefecture, northern Japan. Two major and three minor cpDNA haplotypes were detected from a total of 518 trees representing 63 natural populations that were also divided into at least two genetic clusters based on allele frequencies of seven microsatellite loci. As a result, at least three seed zones were suggested for F. crenata in Miyagi. Furthermore, cpDNA haplotypes of 71 trees representing 18 planted populations showed that half of the plantations were nonlocal and that some plantations have a higher risk of genetic disturbance in adjacent natural forests.

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KEYWORDS:

seed zone; cpDNA haplotypes; population genetics; beech; genetic disturbance.

Phylogeography of *Larix sibirica* and *L. sukaczewii* from Russia inferred from nucleotide variation in the 4CL and 5.8S gene regions

I. Khatab, N. Araki, H. Ishiyama, N. Inomata, A. E. Szmidt

Larix sibirica, L. sukaczewii, L. gmellinii, L. olgensis and L. cajanderi are main larch species in Russia and constitute 40% of its forest. However, there is little knowledge about Larix phylogeography, taxonomy and evolution in Russia, especially about L. sibirica and L. sukaczewii. Based on DNA variation of non-coding region and allozyme variation it has been suggested that genetic variation of Larix species is low. To clarify phylogeography of L. sibirica and L. sukaczewii from Russia we investigated levels and patterns of nucleotide variation of a partial region of the nuclear gene 4-coumarate coenzyme A ligase (4CL) and the 5.8S region including 5.8S rDNA gene and its two internal transcribed spacers (ITS1 and ITS2). We studied three populations of L. sibirica from south of the Ural Mountains and six populations of L. sukaczewii from western Russia. For the 4CL gene nucleotide diversity (pi) was 0.0025 and 14 haplotypes with 12 segregating sites were observed. For the 5.8S region, nucleotide diversity (pi) was 0.0020 and 25 haplotypes with 31 segregating sites were found. In both regions, some haplotypes were shared among all investigated populations. However, their frequencies clearly differed between the two investigated geographical areas. Furthermore, high differentiation was found among the L. Sibirica populations. During Pleistocene western Russia was covered by ice and refugias of Larix were present south of the Ural Mountains. Our results suggest that Larix populations from western Russia have colonized this region only recently. In addition geographic distribution of haplotypes was consistent with classification suggesting that L. sibirica and L. sukaczewii represent two different species.

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KEYWORDS:
phylogeography; Larix; molecular evolution; population genetics; DNA sequence.

Nucleotide variation in allozyme coding genes in *Pinus sylvestris*

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Based on the results of various allozyme and RFLP-studies, it has been thought that pines and other conifers are among the most genetically diverse plant taxa. A high mutation rate together with large effective population size has been thought to result in high nucleotide diversity and thus explain the observed diversity. However, the level of nucleotide polymorphism in Scots pine genes and those of other conifers sequenced so far seems to be comparable to that reported in other plants, not higher as might have been expected. Insertion deletion variation could explain the diversity seen in RFLP-studies but it is not very likely to explain the variation in the coding regions of enzyme genes. Besides high mutation rate, high diversity at the allozyme level could be explained by high recombination rate resulting in many different haplotypes seen as different electromorphs, or by the action of selection maintaining variation. Since only low population differentiation has been observed in allozymes, balancing selection within populations is a possibility. However, by using Ewens-Watterson homozygosity test, we found that only at one allozyme locus (among 13 loci studied in four populations) the frequencies of different electromorphs were consistently more even than expected under neutrality. In order to study the basis of the allozyme variation in Scots pine at the nucleotide level, we have identified five loci potentially coding for allozyme genes. The identity of the loci will be verified by simultaneously determining the enzyme electrophoresis phenotypes and nucleotide sequences from the haploid megagametophyte tissue in a set of heterozygous individuals. The levels and patterns of nucleotide diversity in the loci will be analyzed in a population sample from Finland.

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KEYWORDS:
allozymes; enzyme electrophoresis; nucleotide variation; linkage disequilibrium; balancing selection

Pollen movement and matrix quality; the ecological genetics of fragmentation

***Tonya Lander*, Stephen Harris, David Boshier**

Fragmentation studies are dominated by 'neutral matrix' and 'isolation by distance' models, which derive from Island Biogeography Theory. This theory defines the landscape in terms of 'islands', which contain the only functional habitat, and a surrounding homogenous matrix or 'hostile sea of non-habitat'. The matrix is only a 'distance to be crossed', with decreasing gene flow between island populations as distance increases.

Our study questions this broad-brush approach and investigates the possibility that land uses may vary in the degree to which they present a barrier to, or facilitate, gene flow, depending on the ecological requirements of the organism and the ecological attributes of the matrix. Our study focuses on an endangered endemic tree species from Chile, Gomortega keule (queule; Gomortagaceae) and asks: does pollen travel with equal success between tree populations separated by three different matrix types: agricultural land, pine plantation, and native forest?

The poster describes the main features of this systematic study of the impact of landscape fragmentation on pollen movement using an ecologically explicit landscape instead of a homogenous matrix. Eventual results may have significant implications for; theoretical landscape ecology, the way habitat corridors and biological reserves are conceived of and designed, and our ability to simultaneously manage landscapes for conservation, economic and social value.

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KEYWORDS:
Island Biogeography Theory, habitat fragmentation, pollen flow, matrix permeability, Chilean endemic tree species

Hybridisation dynamics in the white oaks species complex (*Quercus robur, Q. petraea, Q. pubescens* and *Q. pyrenaica*).

O. Lepais[1]**, S. Gerber**[1]

Hybridisation is a common phenomenon in plants species and can have great evolutionary consequences in the fate of species. For example, it has been shown that hybridisation happened during post glacial recolonisation in European oaks species and this mechanism has speed up the colonisation of new land by trees. Current hybridisation between four species of white oak is studied in- and ex-situ. Genetic markers differentiating species (nuclear microsatellites) are used to genotype oaks in a natural forest containing the four species using a multiplex PCR protocol. Results of assignment technics and parentage analysis on seedlings are analysed in order to understand the dynamic of hybridisation in this natural environment. Oaks resulting from interspecific controlled crosses will identify potential genetic incompatibilies between species and hybrid seedlings monitoring is performed to monitor hybrids viability. Ecological and historical data and the spatial distribution of individuals will help to understand hybridising phenomenon in this species complex.

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KEYWORDS:
Quercus, hybridization, gene flow, assignment, parentage analysis

Lineage coincidence of chloroplast and mitochondrial DNA in three Western Mediterranean evergreen oaks

***Unai López de Heredia*[1]; Pilar Jiménez[2]; Carmen Collada[3]; Luis Gil[1]**

Organelle DNA is widely used in phylogeographic studies. Haploid chloroplast and mitochondrial DNA show maternal inheritance in angiosperms but evolve independently. The coincidence and distribution of cpDNA and mtDNA lineages was evaluated on three Mediterranean evergreen oak species (Quercus suber, Quercus ilex and Quercus coccifera). PCR-RFLP analysis of organelle variation showed four lineages (suber¸ i-c I, i-c II and i-c III) with strong geographic structure. The four lineages were independently identified at cpDNA and mtDNA. Chlorotypic diversity was signicantly higher than mitoypic diversity at all four lineages, because of the higher mutation rate of cpDNA vs mtDNA. Introgression is the most likely hypothesis to explain the sharing of lineages among species. Some disassociation between mitotypes and chlorotypes was found within lineage i-c I produced by lineage sorting due to relative age of cpDNA vs mtDNA.

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KEYWORDS:
Quercus, mtDNA, cpDNA, lineage sorting, introgression

Genetic diversity in *Nothofagus nervosa* detected with cpDNA, isozyme and adaptive traits and its implication f or conservation strategies in Patagonian National Parks

Paula Marchelli[1] **& Leonardo Gallo**

Nothofagus nervosa (Phil.) Dim et Mil is a deciduous, anemophilous and anemochoric southern beech endemic to Chile and Argentina in southern South America. It occurs in the Andes and Pacific Coastal Mountains between 35º and 41º S. In Argentina the distribution is narrow and fragmented following the west-east oriented glacial valleys. Due to its valuable wood, N. nervosa was overexploited in the past and several populations were drastically reduced, especially in Chile. As it is usually the rule, the genetic variation of the species was not taken under consideration when protected areas were established. In Argentina, where the whole distribution range of N. nervosa lies within two National Parks, the definition of Evolutionary Significant Units is under consideration and therefore genetic studies have begun. The main objective of the present work is to study the genetic variation of Nothofagus nervosa through different neutral markers and also through adaptive traits such as seedling height in order to contribute with the design of conservation strategies.

Seeds from a total of 20 populations covering the entire geographical range of the species in Argentina were collected. Both uniparentally (cpDNA) and nuclear (isozymes) inherited markers were analysed (eight isozyme loci and two cpDNA fragments (PCR-RFLP)). Seedling height was measure from provenance trials installed in the field.

Relatively high levels of genetic diversity and differentiation were detected with the nuclear markers (AL = 3.38; Ho = 0.16; He= 0.18; $\delta = 0.047$ ($F_{ST} = 0.052$)). Genetic distances between neighbour populations was significantly different indicating high genetic differentiation and probably low levels of gene flow. Three cpDNA haplotypes were identified with the two primer/enzyme combinations scored. Only three populations showed intra-population variation and a high gene differentiation ($G_{ST} = 0.868$) was observed. Moreover, the haplotypes showed a highly structured geographic distribution with a north-south variation. Seedling height at the age of five years was variable among populations ranging from 73.2 to 153 cm (average 115.6 cm).

One population (Hua Hum) showed the highest levels of diversity with both markers, and average values for seedling height. This population was under management since within National Parks some buffer areas are allowed for logging.

Given the results of the present study, the authorities of the National Park Administration changed the protection status of the mentioned population and prohibited logging within it. It constitutes the first case in Argentina where genetic studies were taken into account to decide conservation priorities.

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KEYWORDS:
Patagonian forests, population genetics, conservation, molecular markers, adaptive traits.

Development of a molecular marker based on storage proteins for forest species

***M.A. Martín*, J.B. Álvarez, L.M. Martín**

The storage proteins have shown to be an useful tool as molecular marker of the genetic diversity in Triticeae by its high polimorphism level and the low cost of its analysis.
We are working in the development of this marker in forest species broadleaved and conifers.

It has been stated that the globulin fraction of the storage proteins shows good possibilities in Pinus pinea L. and Abies pinsapo Boiss., whereas albumins can be used in Castanea sativa Mill. and Quercus ilex L.

The storage proteins in sweet chestnut have shown a certain differential genetic integrity among the traditional varieties of Sierra de Aracena (Huelva) and Valle del Genal (Málaga). In addition, this marker is being used to verify the controlled crosses.
In conifers the study of this marker is facilitate by the haploid nature of the reserve tissue where such proteins are accumulated.

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KEYWORDS:
genetic resources, molecular marker.

Geographical pattern of haplotype variation on native stands in Norway Spruce in Austria

***Michael Mengl*, Thomas Geburek, Silvio Schueler**

Norway spruce (Picea abies) is the major and economically most important tree species in Austria, prevailing in approximately 54 % of the Austrian forests. A huge proportion of these forests have been planted, often in unsuitable regions. Those allochthonous populations are especially prone to biotic and abiotic stress. Hence, the identification of autochthonous stands has become an important issue in the context of genetic conservation as well as for the selection of suitable seed stands.

In the present study, we analysed the haplotype intraspecific variation in natural populations by means of a mitochondrial DNA marker. We used the second intron of the nad1 gene, which.contains two polymorphic short tandem repeats. Due to the maternal inheritance of mitochondrial DNA the spatial distribution of haplotypes allows insights into gene flow and artifical seed transfer. The samples were collected in a high density grid of 4 x 4 km in the framework of the National Austrian forest inventory from putatively autochthonous stands. In total 504 trees were screened and 9 different haplotypes could be found. A geographical map of the haplotype variation pattern is presented. This work will be the prerequisite for a future study to identify the intensity of artificial introduction of forest reproductive material.

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KEYWORDS:
Picea abies; mitochondrial DNA; biogeography; genetic diversity.

I-P39

Genetic variation in susceptibility to *Phytophthora cinnamomi* in Spanish natural chestnut populations

Mª Eugenia Miranda-Fontaíña and Josefa Fernández-López

Phytophthora cinnamomi is a pathogen that causes ink disease in European chestnut trees (Castanea sativa Miller) in the humid Northwest of Spain, since 19th century. The objective of this study was to evaluate the variability of Spanish C. sativa populations in susceptibility to P. cinnamomi. The susceptibility of six wild high populations of chestnut was evaluated by an excised stem inoculation experiment under controlled environmental conditions. Six wild high populations of chestnut from six distant sites were selected in Spain with the criteria of representing the Spanish distribution area of C. sativa, including extreme populations regarding climate and geographic distance: 3 sites in the humid climate of Northern region (Lóriga, Requeixo, Nandiello), 1 in continental Northern region (Médulas), 1 site in the Central region (Humilladero) and 1 in the extreme Southern area of distribution (Gaucín). The apex of excised stem 30 cm long were inoculated with P. cinanamomi in February 2006 and the length of lesions produced after 14, 21 and 28 days after inoculation was used as an indicator of P. cinnamomi resistance (low value of length of lesion) or susceptibility. Also the initial flushing after 28 days in greenhouse was evaluated and was correlated with the lesion length. There were significant differences among populations and families for all variables. The populations came from the humid climate of Northern were more resistant than the others, these Northern populations belongs to areas with high presence of P. cinnamomi. The phenotypic correlations among lesion length variables and flushing were low. The individual heritabilities of lesion length variables ranged between 0.39 and 0.50. The results indicate genetic variation in resistance to P. cinnamomi.

Table 1. Influence of Population and Family variation on the length of lesions in mm (LL) produced after 14, 21 and 28 days in mm and flushing (Fl) after 28 days. F values, significance levels of analyses of variance, individual heritabilities, clonal means and Additive coefficients of variation (CVa). Covariate: Flushing.

Source of Variation	df	LL14 (1)	LL21 (1)	LL28 (1)	Flushing (2)
Flushing (covariate) Fl	1	8.80**	45.73***	6.28*	
Population (P)	5	9.28***	11.91***	15.72***	3.89**
Family (Population) F(P)	38	7.74***	6.97***	6.16***	4.56***
Block (B)	4	12.17***	5.94***	8.12***	24.22***
Population*Block (P*B)	20	0.77 ns	0.41ns	0.41ns	1.49ns
Error	2021				
H^2_i		0.50	0.45	0.39	0.28
Mean		26.52	51.89	83.78	1.05
CVa (%)		23.19	24.34	24.47	56.42

Models of ANOVAs: (1) $X_{ijkl} = \_ + Fl28_{ijkl} + P_i + F_{j(i)} + B_k + P*B_{ik} + \varepsilon_{l(ijk)}$; (2) $X_{ijkl} = \_ + P_i + F_{j(i)} + B_k + P*B_{ik} + \varepsilon_{l(ijk)}$
ns= P>0.05; * = P>0.01; ** = P<0.01; *** = P<0.001

Table 2. Classification of populations according to the length of lesions values (LL) in mm obtained with Lsmeans test.

Population	LL14	Population	LL 21	Population	LL 28	Population	Flushing
As Médulas (Le)	33.18	As Médulas (Le)	65.75	As Médulas (Le)	110.47	Gaucín (Ma)	1.30
Gaucín (Ma)	29.46	Gaucín (Ma)	60.13	Gaucín (Ma)	99.65	Lóriga (Co)	1.18
Humilladero (Ca)	27.51	Humilladero (Ca)	54.36	Humilladero (Ca)	87.62	Humilladero (Ca)	1.05
Requeixo (Lu)	24.98	Requeixo (Lu)	48.32	Requeixo (Lu)	77.46	As Médulas (Le)	1.02
Lóriga (Co)	23.81	Lóriga (Co)	45.45	Lóriga (Co)	70.85	Requeixo (Lu)	1.00
Nandiello (As)	21.84	Nandiello (As)	40.39	Nandiello (As)	62.85	Nandiello (As)	0.64

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KEYWORDS:
Castanea sativa, wild populations, ink disease resistance, population differentiation, additive coefficient of variation.

Genetic structure of *Castanea sativa* wild populations from Spain

***Ana Belén Monteagudo*[1], Josefa Fernández-López**

Castanea sativa Mill. is the only European specie of the genus Castanea and is distributed across the Mediterranean region, from the Caspian Sea to the Atlantic Ocean. It is thought that the specie survived in several refuges during the last ice age in Mediterranean Peninsulas. Knowledge about the post-glaciar recolonisation events of European continent from chestnut refuges is still inadequate.

Spanish chestnut has a discontinuous distribution in several patches. The largest continuous area is in the North western region, occupying 70% of the total chestnut distribution area. Isoenzymes were used to examine genetic variability within and among 17 population of wild Spanish chestnut with purpose of design fitted conservation and management strategies. The existence of possible chestnut refuges in Spanish area was checked too.

Allelic frequencies, measures of genetic diversity such as effective number of alleles per locus, expected heterozygosity and percentage of polymorphic loci, F-statistics, D-statistics, gene flow, Nei´s genetic distances and AMOVA were calculated.

According to the results Spanish wild populations shows good levels of genetics variations. The exception were four population with low levels of genetic variation and inbreeding effects. The D-statistic and cluster analysis showed a structure of subdivided populations with limited gene flow between populations and random genetic drift with local differentiation.

The Astur-leonesa zone and the South western of Galicia were pointed as possible refuges of chestnut during glaciations period.

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KEYWORDS:
Spanish chestnut; wild populations; genetic variation; isozymes; chestnut refugium.

Paternity analysis of two Dipterocarp species reveals high selfing rate, paternity heterogeneity and hybridization between species

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Mass flowering event is well known in tropical forest of south eastern Asia, which many tree species without taxonomic relationship held flowers together and might be caused by aperiodical climate change as trigger. Mass flowering event affects insect community growth, subsequently ecosystem of tropical forest. In terms of tropical forestry, Dipterocarp species is the most important taxon for timber production in south eastern Asia. Therefore, understandings of paternity pattern and pollen flow via insects are essential to conserve tropical forest ecosystem and to develop a suitable logging system. Here, we present the paternity analysis for two Dipterocarp species, Shorea leprosula and S. parvifolia in Pasoh forest reserve, Negri Sembilan, Malaysia. We collected DNAs from mature trees (>30cm DBH) and seeds and seedlings from 13 maternal trees in two flowering years, 2001 and 2002. Then ten microsatellite markers' genotypes were obtained for all samples and used for paternity analysis. Selfing rate for seeds samples was relatively higher than it for seedlings, which might be in consequence of inbreeding depression during seed germination to seedling stage. A number of paternal trees detected in each maternal tree's progeny is one to seven, this number is very small with considering total number of mature trees. Each maternal tree received pollen from particular paternal trees. The paternity analysis revealed that two S. leprosula progenies were transmitted pollen from S. parvifolia trees. This is direct evidence of hybridization between the two species. These results are first step to understand pollen flow pattern of Dipterocarp species, and we will develop new criteria for logging system with considering pollen flow pattern of Dipterocarp species to conserve tropical forest ecosystem.

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KEYWORDS:
Dipterocarp, tropical forest, paternity, microsatellite, hybridization

Evaluation of the power of assignment methods on chloroplast microsatellites for provenance identification of reforested stands

Miguel Navascués[1]

Provenance identification with molecular markers can be a useful tool to control the commercialization of forest reproductive material and to assess the origin of historic reforested stands. Currently, there is a wealth of genetic data based on chloroplast microsatellites (cpSSRs) for conifer species; thus, the utility of cpSSRs for origin identification would be highly beneficial. For this work power of several previous statistical methods for the identification of the population origin of individuals and groups of individuals has been explored. These methods can be classified in three groups: distance based methods, likelihood based methods and methods based on differences in frequencies (only applicable to groups of individuals). An additional likelihood based assignment method was introduced that also accounts for genetic distances. For assessing the performance of the different methods data for linked microsatellites was simulated with SIMCOAL for a wide range of scenarios (different levels of genetic differentiation, sample size and number of loci).

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KEYWORDS:
chloroplast microsatellites, assignment methods, reforestation, provenance identification, SIMCOAL

Pan-Eurasian variation in mitochondrial DNA reveals multiple distant glacial populations and the postglacial history of Scots pine (*Pinus sylvestris*)

***Krassimir Naydenov*[1]*, Sauphie Senneville[2], Jean Beaulieu[2,3], Francine Tremblay[1] and Jean Bousquet[2]**

Scots pine (Pinus sylvestris L.) is an evergreen conifer with a wide natural range covering much of Eurasia. The genetic structure related to the biogeographical history and postglacial colonization of this species was investigated using novel mtDNA polymorphisms detected in the intron 1 of the gene nad7 for 54 natural populations spanning from western Europe to eastern Asia. Among the 986 trees analysed, four distinct mitotypes were detected based on two size variants for nad1 intron B/C and three novel size variants for nad7 intron 1. The average number of mitotypes per population was 1.50, and the mean haplotype diversity (H) was 0.141. Population differentiation was high ($G_{ST} = 0.657$) and the distribution of the mitotypes was geographically highly structured. Spatial analysis of molecular variance (SAMOVA) identified four homogenous groups of extant populations that presumably represent genetically distinct glacial populations: a small group mostly restricted to the Iberian Peninsula, a second small group confined to Asia Minor, a much larger group mostly restricted to Northeastern Europe including the Baltic region, and a cosmopolitan group present in much of Europe and Asia. A large suture zone was detected in central Europe and part of Scandinavia between the two last groups. Together with evidence from the fossil record, the postglacial biogeographical history of Scots pine was inferred. The results suggest a complex history with some refugia shared with other plant species and others contributing little to the current distribution of Scots pine. The implications for our understanding of Eurasia Holocene recolonization are discussed.

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KEY WORDS:

Glacial refugia, biogeographical history, mitochondrial DNA, phylogeography, Pinus, population genetic structure, suture zones

Differentiation and hybridization between *Quercus alnifolia* Poech and *Quercus coccifera* L. in Cyprus. A study with DNA microsatellite markers

***Charalambos Neophytou*[1,2], Filippos Aravanopoulos[3], Aikaterini Dounavi[1]**

The holly oak (Quercus coccifera) and the golden oak (Quercus alnifolia) are the only two indigenous evergreen oak species of Cyprus, mostly occupying different habitats. Sympatric populations rarely occur, while interspecific crossings are possible. In previous studies morphological traits and isoenzyme markers were used to differentiate the two oaks, however no species-specific markers were found.

Our work aims at studying the intraspecific genetic variation and interspecific hybridization by using nuclear microsatellites. Two pure parental species populations and one sympatric stand were sampled. Genomic DNA was extracted from vacuum dried leaves. Nine nuclear microsatellites developed from other oak species were initially tested.
Five out of the nine microsatellites tested were successfully amplified but only three were polymorphic. Sixteen alleles were detected for the locus ssrQpZAG9, eight for ssrQpZAG96 and seventeen for MSQ13. The described set of microsatellite markers can distinguish the parental species since it contains private alleles. Furthermore, our results indicate distinct genetic structures between the two species and provide evidence of a limited genetic introgression, in concordance with results from a multivariate analysis of morphological traits.

This is the first study using molecular markers in the above species conducted thus far.

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KEYWORDS:
nuclear DNA microsatellites; private alleles; genetic introgression.

Adaptive genetic variation of in a latitudinal cline in Scots pine: Growth rhythm traits and curves analysis.

Eduardo Notivol[1], **Leyre Corcuera**[1], **Ricardo Alía**[2]

Pinus sylvestris L. (Scots pine) has the widest distribution of pine species and the populations are locally adapted to very different environmental conditions. Adaptive traits such as those related to growth are optimal for understanding adaptation of populations to local conditions in wide distributed forest species. The timing of growth during the third growing period of families within nine populations from the latitudinal cline of the distribution range was conducted. Individual growth curves were fitted and a set of variables related to growth rhythm was obtained for genetic analyses. Pooled heritabilities across populations where high for most of the traits and ranked from 0.20 to 0.68 for the different traits and population differentiation for growth variables were quite variable (Q_{ST}: 0.09-0.25). The results show a high additive variance for most of the growth variables. Familiar variance represents between 4% and 14% of the total variance for the set of all the traits in all the populations. Even if no general patterns of additive variance was found, the high additive genetic variance obtained in our study for many different traits suggest that additive genetic variance is not the limiting factor for adaptation to a new optimum within much of the range for these traits. Changes in means, additive genetic variances and additive genetic coefficient of variation by population are also presented.

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KEYWORDS:
Adaptive variation, growth curves, populations

Effects of population density on pollen flow in the wind-pollinated tree, *Fagus crenata*

Naoto Numano[1] **and Yoshihisa Suyama**[1]

Past researchers have shown that forest fragmentation can cause reduced seed production and genetic erosion in future generations. To evaluate the effect of forest fragmentation on pollen flow, seed set and genetic diversity of resulting seeds were compared among nine trees of Fagus crenata located at various population densities (1-34 adult trees / ha) in a ca. 500 ha study plot in northern Japan. We collected a total of 9453 seeds and counted the numbers of mature, immature, and insect-attacked seeds. Ninety-six mature seeds from each tree were used for microsatellite analysis. As expected, seed set and population density are positively correlated. But contrary to our expectation, genetic diversity of produced seeds was high at low population density. Paternity analysis revealed relatively high number of pollen donors and long distance pollen dispersal at low population density. In addition, outcrossing rate was high (95-100 %) even for low-density trees. Although pollen limitation was suggested by seed set data, pollen flow over long distance was effective in the low-density populations of F. crenata in the study plot.

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KEYWORDS:

genetic diversity; forest fragmentation; gene flow; paternity analysis; microsatellite.

Dispersal and *installation patterns* of the forest invasive black cherry in two different landscapes in Belgium.

Marie Pairon[1]**, Anne-Laure Jacquemart**

Dispersal dynamics presumably influence plant processes such as colonization of new habitats and are therefore of tremendous importance in understanding the underlying mechanisms of invasions. Introduced at a large scale and massively planted by foresters in the 19th century, black cherry (Prunus serotina Ehrh.) rapidly filled the gaps between the plantings and established dominant thickets. It is thought to impede the rejuvenation of forests and to reduce the plant richness under its canopy. Belonging to the Rosaceae family, black cherry produces small cherries nearly every year. The bulk of the seed crop falls to the ground in the vicinity of the parent tree but some seeds are dispersed by mammals and birds. Because long distance dispersal events are of major importance in explaining the spread of invasive species, and because these events are hard to monitor by direct means, 7 polymorphic microsatellite markers were chosen to describe the dispersal and installation mechanisms of the species in Belgium. Two different scales were chosen in two different sites (S=4 km_ and 25 km_). The bigger trees (CBH > 130 cm) were mapped and sampled. Isolation by distance patterns were studied and assignment tests were performed to evaluate the contemporary gene flow between the initial known introduction points and the newly created small demes situated at distances ranging from 1 – 7 km away from the point sources. The implication of the results for explaining the invasive success of the species are discussed.

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KEYWORDS:

invasive species; dispersal; population genetics; isolation by distance; microsatellite markers.

I-P48

Linking gene polymorphism to wood property variation in maritime pine

Camille Lepoittevin[1,2a], Pauline Garnier-Géré[1], Emmanuelle Eveno[1], Luis Cancino[1], Annie Raffin[1], Denilson da Silva Perez[2b], Luc Harvengt[2a], Christophe Plomion[1]

Wood properties are known to vary among genotypes within species. This variability is heritable and thus presents an opportunity to select for improved wood properties i.e. superior product quality. Such selection is currently hampered by costly chemical and technological assays and the necessity to wait until the trees are nearly mature to evaluate wood properties. Application of modern genomic sciences to identify the genes controlling these properties, should increase selection efficiency and/or reduce the time and costs associated with measuring such properties, by providing early selection criteria. In this poster we will describe and present preliminary results on the quantitative genetics aspects of an ANR funded project entitled "**Genomics of wood formation and molecular tools for breeding wood quality in maritime pine": ie**. i/ the study of the landscape of nucleotide diversity in candidate genes previously identified in maritime pine or in the model plant species Arabidopsis thaliana, and ii/ the relationships between naturally occurring nucleotide polymorphisms with phenotypic variability of wood quality traits measured in experimental designs of the maritime pine breeding program..

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KEYWORDS:

Wood quality, maritime pine, candidate genes, association study, nucleotide polymorphism

Short distance dispersal patterns of pollen in California valley oak, *Quercus lobata* (Fagaceae)

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Delphine Grivet[1]**, Jeanette Papp**[7]**, and Peter E Smouse**[8]

Pollen dispersal, a critical genetic process for plant populations, has two components: short distance dispersal (SDD), which determines the neighborhood size (sensu Wright 1943), and long distance dispersal (LDD), which provides connectedness among populations. We investigated SDD in a low-density stand of Quercus lobata with likelihood parentage analysis, assigning paternity of 474 progeny from five seed-trees to 120 of 161 candidate pollen donors, within a 250 m radius of the focal seed-tree. A few trees close to the seed tree sired a disproportionate number of progeny, while most candidate pollen donors sired a few seeds. The overall mean observed pollination distance was 114.1 m (± 69.8 SD). Pollen dispersal was better described by an exponential power or an inverse power function than by a negative exponential function. We found that inclusion of directionality in non-linear models of pollen movement improved the “fit” of the model slightly. The angle of directionality did not correspond to the predominant wind-direction, but casual observations suggest that local vegetative structure might have had an impact. Our analysis of SDD indicates that a few highly successful pollen donors, proximal to the seed trees, account for small local neighborhoods, sensu Wright (1943). Simultaneously, the reasonably good fit of both exponential power and inverse power models reveals a fat tailed dispersal distribution, compatible with potentially substantial long distance dispersal in Valley oak. However, only studies that can capture a larger fraction of LDD will allow us to distinguish (compellingly) between these dispersal functions.

Wright S (1943) Isolation by distance. Genetics, **28**, 114-138.

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KEYWORDS:

Dispersal functions; paternity analysis; pollen movement; SSR markers; wind pollination.

Effects of biotic stress on Norway spruce seed quality, antioxidant content and genetic composition in 2 Slovenian seed stands

Marjana Pu_ko[1], **Cvetka Ribari_ Lasnik** [2], **Gregor Bo_i_** [1], **Monika Konnert**[3], **Hojka Kraigher**[1]

During the last mast year Norway spruce was heavily attacked by bark beetles. Because the nutritive tissue in conifers is derived entirely from the female parent the effect of biotic stress could influence the seed quality. The presence of biotic stress was detected through the visual signs caused by infestation with bark beetles and fungi and confirmed with slightly higher although not statistically significant antioxidant content in needles of non-vital trees. Together 40 trees belonging to vital and non-vital subpopulations from two Slovenian populations (Idrija, Pohorje) were analysed for seed quality, _-tocopherol and ascorbic acid content in one year old and current year needles. STS and EST markers were used in an attempt to find genetic differences between vital and non-vital trees. The genetic differences between the populations of spruce and their vital and non-vital subsets were relatively small for the gene pool studied. Statistically significant differences were observed in Idrija population for the number of germinating seeds per cone, weight of 1000 seeds and weight of seeds per cone. No solid evidence against the use of seed derived from sanitary fellings due to biotic damages for production of forest reproductive material in the studied populations was found.

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KEYWORDS:
Picea abies (L.) Karst., biotic stress, seed quality, antioxidant content, genetic structure

Characterization of genetic variation within and among populations of *Gonystylus bancanus* (ramin) in Peninsular Malaysia

Fatma Nadiah Abd Hamid[1], Wickneswari Ratnam[1]*, Chuck Cannon[2]

Genetic variation within and among five natural populations viz. Pekan 1 and Pekan 2 in Pekan Forest Reserve, Pahang, Nenasi in Nenasi Forest Reserve, Pahang, Air Hitam in Air Hitam Utara Forest Reserve, Johor and Belara in Belara Forest, Trengganu of Gonystylus bancanus (ramin) representing the three states of Peninsular Malaysia where this species is still found was investigated using Direct Amplification of Length Polymorphism (DALP) analysis. About 24 – 38 inner bark tissue samples from mature ramin trees were collected from each population. Six DALP primers (DALP221, DALP231, DALP232, DALP233, DALP234 and DALP235) used in this study yielded a total of 316 loci of which 81% (257 loci) were polymorphic for the 156 samples analysed. Shannon's diversity index (H_o) estimated for each population was not significantly different from each other with values ranging from 11.376 to 13.173. On average, only 13% of the genetic variation occurred among populations, with the variation within populations being high (87%). The six DALP primers differed in their capacity to detect genetic variability among and within populations. DALP221 detected the highest variation among populations (0.404) whereas DALP235 detected the lowest variation among populations (0.049). DALP232 detected the highest variation within populations (H_{pop} = 15.346) and DALP221 detected the lowest variation within population (H_{pop} = 8.199). The high genetic variation within populations and low genetic differentiation among populations imply high levels of outcrossing within populations and extensive gene flow among populations. The understanding of the patterns of genetic variation of ramin, both within populations and among populations in Peninsular Malaysia, can be utilized in developing conservation and sustainable management strategies for this commercially exploited peat swamp species.

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KEYWORDS:
genetic variation, DALP analysis, Gonystylus bancanus (ramin), conservation, sustainable management

Comparison of *Pinus pinea* L. populations' genetic structure assessed by AFLP and retrotransposon-based SSAP

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We compared the genetic variation of seven Portuguese Pinus pinea L. (stone pine) populations using retrotransposon-based sequence-specific amplification polymorphism (SSAP) and amplified fragment length polymorphism (AFLP). The PCR primers used in the SSAP technique were designed from the long terminal repeat of P. pinaster (PpRT1) together with a subset of three selective MseI primers. In order to directly compare the SSAP with the AFLP marker system, the same pre-amplification PCR products produced with the EcoRI and MseI were used in the both experiments. The 232 SSAP loci (derived from the LTR retrotransposons) and the 132 AFLP loci were used to screen 105 trees, but the SSAP approach produced a higher number of polymorphic fragments compared to the AFLP one (75.4% and 56.8%, respectively). The differentiation levels were slightly higher for SSAP than for AFLP (F_{ST} = 10.5%, for SSAP and 7.4% for AFLP), and both significantly different from zero, $P<0.01$. The levels of the average within-population genetic diversity found with the two types of marker were similar, and, also, slightly higher for SSAP than for AFLPs (0.2666 and 0.2280, respectively). Even though the values of genetic differentiation are high, for what is usual in outcrossing long-lived species, the genetic variation was found mainly within populations. The populations that displayed the highest and the lowest genetic diversity levels were the same for both markers (Monte Novo: $H_{e(SSAP)}=0.2802$ and $H_{e(AFLP)}=0.3270$; Ponte Lima $H_{e(SSAP)}=0.2541$ and $H_{e(AFLP)}=0.2820$, respectively). A principal component analysis was performed based on Nei's genetic distance after Lynch and Milligan (1994), and the percentage of the total variation explained with the two first axes was 37% and 23% with both markers. The populations did not cluster according to the provenance regions were they were sampled, and no coherent geographic pattern was found with both markers, but they have showed similar populations' clustering.

In this study SSAP generated more polymorphic fragments and higher estimates of genetic diversity than AFPL did, due, probably, to the higher mutation rate of retrotransposition relative to base mutation. Nevertheless, congruence was found between estimates obtained with both markers, which is very interesting, for, in general, SSAP markers have lower costs compared to AFLPs, and they might be an interesting system marker alternative, when higher resolution is requested.

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KEYWORDS:

retrotransposons; sequence-specific amplification polymorphism; amplified fragment length polymorphism; stone pine; genetic structure

I-P53

SEEDSOURCE: The role of gene flow and selection in population delimitation.

Paul D. Rymer[1], **Stephen A. Harris**[1], **David H. Boshier**[1]

A fuller understanding of population structure, delimitation and adaptation can provide a rational basis for the collection of locally adapted and genetically diverse seeds for planting and natural regeneration. The key scientific question is how gene flow and selection interact to influence population delimitation. The relationships between genetic diversity, habitat heterogeneity and the scale of adaptation in trees are complex. Gene flow may counteract even fairly strong selection, preventing formation of locally adapted populations. However, very strong environmental variation (hence selection pressure) may produce adaptive differences over short distances, despite continued high levels of gene flow.

The poster describes ongoing research to unravel the opposing forces of gene flow and selection, as part of an EU project (SEEDSOURCE). We are studying levels of genetic diversity, gene flow, and adaptive variation in Bombacopsis quinata (a widespread long-lived pioneer neotropical timber tree that occurs in a range of environments with disparate water availability). Chloroplast and nuclear microsatellites will be used to determine neutral genetic variation and estimate gene flow across the species range and in contrasting landscapes, both natural and human modified (e.g. forest fragments, isolated trees in pastures). Single Nucleotide Polymorphisms (SNPs) in candidate genes related to water stress responses, well characterised in model plants (e.g. Arabidopsis and Populus), will also be evaluated to study adaptive variation in populations sampled across the species geographic and ecological range. Population choice will be informed by phylogeographic structure and climatic distribution. The availability of SNPs within adaptive genes that associate with major ecotypic differentiation may not only provide insights into the molecular basis of adaptation and selection, but also facilitate the development of specific ecotypic markers for monitoring translocation and aid marker assisted breeding during domestication.

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KEYWORDS:

Adaptive variation, genetic structure, habitat fragmentation, local adaptation, mating patterns

Molecular variation and the colonization history of northern Scots pines

Matti Salmela[1], **Outi Savolainen**[1], **Sheila Hicks**[2], **Tanja Pyhäjärvi**[1]

Scots pine has the widest distribution of all conifers, covering a wide area in Eurasia from western Scotland to eastern Siberia. The distribution of European forest trees has been affected by past changes in climate, as a large part of the species ranges were covered by ice during the last glacial maximum (LGM), after which the species re-colonized the northern part of the continent from southern refugia. Furthermore, according to recent macrofossil and genetic data, populations of cold-tolerant species have possibly existed in central and eastern parts of Europe during the LGM.

Knowledge about the post-glacial colonization routes of Scots pine is required in order to understand the genetic background of its adaptation to various environmental and climatic conditions. Previous studies have focused on the western European populations, and the history of northern pines has remained unclear. Our goal is to study the possibility of colonization events from the east and putative admixture in northern populations. We want to interpret the genetic data in conjunction with paleoecological data, such as pollen accumulation records, from northern Russia and Scandinavia.

We have studied molecular variation of northern Scots pines in 13 populations from Sweden, northern Finland and the western part of Russia using two mitochondrial DNA and four nuclear microsatellite markers. Non-recombining mitochondrial DNA is used for studying colonization history and biparentally inherited nuclear markers provide information about the relative amount of genetic variation among populations.

Preliminary results show that mitotype c was most abundant in northern Finland and northwestern Russia, while mitotype a dominated in Sweden and the easternmost sampled Russian populations.

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KEYWORDS:
colonization; last glacial maximum; microsatellite; mitochondrial DNA variation; refugium

I-P55

Clonal structure of three wild Populus tremula L. populations in Spain

Rosario Sierra-de-Grado[1]**, Inés Villamediana**[1]**, Josu Ciriza**[1]**, Javier Villar**[1]**, Dolores Cristóbal**[1]**, Nikoaos Nanos**[1,2]**, Pablo Martínez-Zurimendi**[1]

Populus tremula L. has a remarkable ability to sprout from roots, which allow it a clonal propagation in nature. In Spain, the Southwestern limit of its geographical distribution, P. tremula forms small populations (generally less than 1 ha). The spatial distribution of the clones in three wild populations of P. tremula L. in Northern Spain was studied. The populations were chosen in three different stages of development (young, mature and old), in order to compare clonal composition and genetic diversity. Based on previous works, the expected trend was fewer and more unbalanced distributed clones in the older populations. In each population, all the trees with DBH bigger than 3 cm were mapped. Then, the age and genotype was identified in 90 to 106 evenly distributed trees per population. The genotype was identified based on seven isozyme systems. Genetic diversity parameters were calculated in each population. Genotype and age of the remaining trees were interpolated by geostatistical methods. A phalanx strategy of clonal growth was found in all the populations. There were clones expanded near 200 m, and in the older population, there were clones that could be more than 250 years old. In the supposedly younger population, the genetic diversity parameters were lower than expected. This fact can be explained by the last decade's history of the population, as it is a post-perturbation population instead of a new or first-stage one. Possible sexual reproduction inside the populations, pattern of sprouting and competition among clones process are discussed.

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KEYWORDS:

isozymes; geostatistical analysis; genetic diversity; clonal competition; phalanx strategy.

Spatial genetic structure in two contrasting wood formations of *Quercus ilex* L. and *Q. suber* L. Effects of history and management.

Zaida Lorenzo, Concetta Burgarella, *Álvaro Soto*, Luis Gil.

An appropriate knowledge of reproductive patterns is needed for the sustainable management of forest. This is especially true in the case of jeopardised formations such as cork oak (Quercus suber L.) and holm oak (Q. ilex L.) parklands, known as “dehesas” in Spain. These are anthropised formations, created and maintained for the interest of the activities carried out in the clearings and under the canopies, such as cropping and grazing; remaining trees provide acorns, very esteemed for pork feeding. Iberian dehesas are nowadays threatened by the lack of regeneration, mainly due to the excess of grazing and insolation, and the decay of aged adult trees, and cannot rely exclusively on their consequently decreasing resprouting ability. Here we present a first approach to this issue from a genetic point of view in two contrasting mixed stands: a dehesa in Toledo, Central Spain, and a more dense wood in Gerona, North-Eastern Spain. Spatial genetic structure due to limited dispersal has been checked in both cases and inferences about gene dispersal have been performed, according to the isolation by distance model.

Noticeable differences have been detected between sites and species: No significative genetic structure have been detected in the Gerona plot for none of the species; conversely, in Toledo, limited effective dispersal leads to familiar structures in cork oak, up to 70 m. No matter their similar ecological roles in dehesas, no genetic structure has been detected for holm oak. These results suggest a very effective dispersal for holm oak, with a Sp statistic (which allows the comparison among species, no matter the sampling scheme) of 0.0044 (0.0215 for cork oak) and a local gene flow estimated between 50 and 90 m.

Results are discussed taking into account the ecological behaviour of the species and the history of these woods. Conclusions are drawn on the evolution of current dehesas and management practices required for their appropriate regeneration, from a genetic point of view.

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KEYWORDS:
Land management, Mediterranean open woods, Regeneration, Spatial Genetic Structure,

Landscape-scale genetic structure in *Fraxinus excelsior*: gene-flow within local valley systems and the genetic consequences of natural or artificial regeneration

Polly Spencer-Vellacott[1]**, Christine M. Cahalan**[1]**, and Chris G. Gliddon**[2]

Current interest in genetic conservation, and hence genetic diversity in native tree species, is leading to changing attitudes in forestry. When planting native species in multi-purpose woodlands, it is now important to consider genetic diversity, with possible implications for adaptation to present and future conditions. Fraxinus excelsior is a common forest tree in Britain. It is wind-pollinated and the seeds are also dispersed by wind. The aims of this study are to quantify the genetic diversity and gene flow at a variety of geographical scales within Wales using microsatellite markers. Leaf samples were collected from two semi-natural valley woodlands in North Wales (Clwyd) and three in South Wales (the Gower Peninsular). In addition, different generational cohorts were sampled in one population in North Wales. Samples were collected from the mature trees, the naturally regenerating seedlings, seed collected from the population and seedlings grown from that seed. The aim is to determine whether the method of regeneration affects the genetic diversity of the population. Preliminary results show a high level of diversity but little differentiation between populations

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KEYWORDS:
Population genetics, Fraxinus excelsior, genetic diversity, regeneration, management

Construction of high-resolution chloroplast haplotype map in *Fagus crenata* for phylogeography and conservation.

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Economically valuable hardwood such as Fagus crenata, Zelkova serrata, and Quercus species have long been exploitatively harvested as timber woods from natural populations. With evident decline of natural resources of those hardwood species and changes in social attitude to the environmental issues, afforestation of the hardwood species have been increasing during the last decade in Japan. However, there is no regulation regarding the seed transfer zones for those species, as they have not long been seen as forestry tree species. Therefore, geographically extensive seed transfer has been common in Japan, and it may cause unintended unfavorable consequences of genetic mixing. To conserve the genetic structure of these species in nature, it is indispensable to clarify phylogeography of the targeted species, and to establish the seed transfer zones.

Fagus crenata is one of economically valuable hardwood species, and its natural distribution in Japan is widespread, from Kuromatsunai Lowland in Hokkaido to Mt. Takakuma in Kyushu in Japan. Fujii et al. (2002) previously studied 45 populations covering the species' range using genetic variation of the non-coding region between the trnL (UAA) 5'exon and the trnF (GAA) and the trnK (UUU) region, and found 13 cpDNA haplotypes. To enhance the phylogeographic resolution potential, we 1) collected more samples extensively, 2) explored further genetic variation on chloroplast genome, and 3) constructed high-resolution haplotype map of F. crenata. We sequenced eight regions of large single copy such as the non-coding regions between trnQ (UUG) and trnS (GCU) and between trnD (GUC) and trnT (GGU), and newly found 30 SNPs and an Indel. We investigated 365 individuals from 98 natural populations using five Indels and 33 SNPs, and found that cpDNA haplotypes distributed in a geographically-structured way.

In order to clarify the relationships between haplotype and quantitative traits, we also investigated the phenotypes of trees collected from different geographic origins in a common garden. We investigated the bud-flush phenology and leaf area of each tree, and surveyed their chloroplast haplotype. These relationships will contribute to understand the adaptation and influence of afforestation in F. crenata.

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KEYWORDS:

chloroplast haplotype map, conservation, Fagus crenata, phylogeography, single nucleotide polymorphisms (SNPs)

Heterogeneous pattern of pollen flow and mating success in a small, isolated population of *Picea glehnii* Masters in Mt. Hayachine, northern Japan.

Motoshi Tomita[1], **Yoshihisa Suyama**[1], **Takeshi Seki**[2] **and Hisashi Sugita**[2]

In small populations, individual mating success is often inconsistent with theoretically predicted models (i.e. dispersal kernels) because of spatially restricted mating neighbors, strong pollen limitation, or heterogeneous fecundity of individual adult trees. The pattern of pollen flow and mating success was investigated in a small population of Picea glehnii Masters to address following questions: (1) how heterogeneous is the pattern of pollen flow and mating in a small population? And (2) what factors affect individual mating success; spatial distribution of adults, fecundity, and genetic relatedness? In 2005, we counted the number of seed cones and number of primary branches with pollen cones of adult trees and sampled 10 cones from 15 trees. Filled seed ratio for each sampled tree was highly variable but generally low, suggesting pollen limitation. We conducted paternity analysis for 96 seeds per each mother tree, then analyzed spatial pattern of pollen flow, and examined factors contributing to individual mating success.

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KEYWORDS:
paternity analysis; microsatellite marker; Sakhalin spruce; spatial heterogeneity; relict.

Needle proteome reference map of *Pinus radiata* D. Don.

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Proteome analysis is a powerful tool in functional characterization, physiological studies and also could help to define markers for family or individual selection in upgrading programs. Before to obtain conclusive data by comparative analysis, a reference proteome for each species and tissues must be defined. In this work the Pinus radiata mature needle subproteome is first presented, being also the largest subproteome defined for Pinus genre.

Full developed needles (13 months old) of ten different Pinus radiata mature field trees were taken for this analysis. Proteins were extracted using a TCA-Acetone precipitation based method, focused in IPG strips (17 cm , pH 5-8 linear gradient), resolved in 13% poliacrylamide gels and coomasie stained. Images were acquired with a GS-800 densitometer and analysed with PDQuest 7.1 software.

Analytical and biological variation over 170 spots was studied, obtaining CVs of 24% and 32% respectively. Spots with high variability were also defined. Due to the poor database coverage for protein and DNA sequences in P. radiata, peptides were identified by de novo sequencing, by LC-MS/MS, and BLAST similarity searching. This process led to identify about 80% of spots.

Result constitutes the ground reference map of our scientific programme aimed to identify protein changes along P. radiata ageing process and its regulation at genomic level.

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KEYWORDS:

Pinus radiata, proteome, needle, de novo sequencing, LC-MS/MS.

What maintains hybrid genotypes in *Populus*? A detailed survey in *Populus* × canescens, a natural hybrid between *P. alba* and *P. tremula.*

***Marcela van Loo*[1], Jeff A. Joseph[1], Berthold Heinze[2], Mike F. Fay1 & Christian Lexer[1]**

Natural hybrid zones as active sites of evolutionary change are often viewed either as localities in which selection against hybridization leads to strong barriers to gene exchange, or as sources of new recombinant types or species. Numerous studies report that hybrid genotypes sometimes experience a fitness advantage relative to their parents, but the question as to how these fit hybrid genotypes are maintained in natural populations in the face of recombination remains contentious. Here, we address this issue for a natural hybrid zone between two diploid, ecologically divergent European tree species with sexual/asexual reproduction, Populus alba L. (white poplar) and P. tremula L. (European aspen). In more detail, we concentrate on P. × canescens (grey poplar, the natural hybrid,) and its sympatric backcross parent P. alba from a Central European hybrid zone. In both taxa, Spatial Genetic Structure, clonality and their ecological amplitudes were assessed and compared. We discuss implications of our data for persistence of P. × canescens, and we comment on the evolution of reproductive isolation and invasiveness in natural Populus hybrids.

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KEYWORDS:
hybrid zone; Populus; spatial genetic structure; clonality; microsatellites.

I-P62

Range-wide phylogeography and delineation of gene zones in *Pinus pinaster* Ait. based on chloroplast microsatellite markers

Gabriele Bucci[1], Santiago C. González-Martínez[2], Christophe Plomion[3], Maria Ribeiro[4], Federico Sebastiani[5], Ricardo Alía[2], Giovanni G. Vendramin(1)

Some 1339 trees from 48 stands spread over the whole range of Pinus pinaster have been characterized by 5 chloroplast microsatellite loci, detecting a total of 103 distinct chloroplast haplotypes. Frequencies for the most abundant 16 haplotypes were spatially interpolated over a lattice made by 430 grid points covering the whole species range. Fitting of spatially interpolated values on raw haplotypes frequencies at the same geographic location was tested by regression analysis. A range-wide “diversity map” based on interpolated haplotypes frequencies was then obtained, allowing the identification of an “hotspot” of intraspecific biodiversity in central and southeastern Spain, and a wide area of low haplotypic diversity located in the western Iberian peninsula. Principal component analysis carried out on haplotypes frequency surfaces allowed the construction of a color-based “synthetic” map of the first three PC components (variance accounted for: 74.29%), enabling the detection of the main range-scale genetic trends, and the identification of three main gene pools for the species: (a) a “south-eastern” gene pool, including southeastern France, Italy, Corsica, Sardinia, Pantelleria, and northern Africa; (b) an “atlantic” gene pool, including all the western areas of the Iberian peninsula; (c) a “central” gene pool, located in southeastern Spain. Cluster analysis carried out on grid point frequency values revealed the existence of 8 major clusters (“gene zones”), whose genetic relationships were related with the evolutionary history of the species. Using discriminant analysis on the above grouping, 97.08% of the grid points of the lattice were correctly reclassified into the assigned gene zone after cross-validation. All genetic boundaries between adjacent gene zones were statistically significant after analysis of molecular variance. The application of the above results in the field of conservation of genetic resources and breeding are discussed.

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KEYWORDS:
cpSSR, maritime pine, geostatistics, breeding zones, haplotype diversity map

Analysis of spatial genetic structure in an expanding *Pinus halepensis* population reveals development of fine-scale genetic clustering over time

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We analyzed the change of spatial genetic structure (SGS) of reproductive individuals over time in an expanding Pinus halepensis population. To our knowledge, this is the first empirical study to analyze the temporal component of SGS by following the dynamics of successive cohorts of the same population over time, rather than analyzing different age cohorts at a single time. SGS is influenced by various factors including restricted gene dispersal, microenvironmental selection, mating patterns and the spatial pattern of reproductive individuals. Several factors that affect SGS are expected to vary over time and as adult density increases. Using air photo analysis, tree-ring dating and molecular marker analysis we reconstructed the spread of reproductive individuals over 30 years beginning from five initial individuals. In the early stages, genotypes were distributed randomly in space. Over time and with increasing density, fine-scale (<20m) SGS developed and the magnitude of genetic clustering increased. The SGS was strongly affected by the initial spatial distribution and genetic variation of the founding individuals. The development of SGS may be explained by fine-scale environmental heterogeneity and possibly microenvironmental selection. Inbreeding and variation in reproductive success may have enhanced SGS magnitude over time.

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KEYWORDS:
spatial genetic structure, spatial autocorrelation, Pinus halepensis, population expansion, microsatellites

Ancient DNA of *Fagus orientalis* complex in Italy: an unexpected distribution inferred from 45,000-year-old DNA samples

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Traditional classification of beech in Western Eurasia recognizes two main species, Fagus sylvatica and Fagus orientalis, distributed in western, central and southern Europe and Middle East respectively, together with two intermediary types, Fagus moesiaca and Fagus taurica. Despite extensive palynological, morphological and phylogeographical analyses, the phylogenetic relationships of the Western Euroasiatic Fagus spp. remain unresolved so that is still controversial whether F. sylvatica and F. orientalis are really separated. In this work we aim at testing if the trnL-trnF chloroplast DNA (cpDNA) region, that has been previously proved efficient in discriminating different Quercus taxa, can be employed to distinguish the Fagus species. To cover the wide distribution range of the Western Euroasiatic beech, 86 populations from 4 species (F. orientalis, F. sylvatica, F. taurica and F. moesiaca) were sampled and sequenced for the trnL-trnF region. Furthermore Fagus crenata (2 populations), Fagus grandifolia (2 populations), Fagus japonica, Fagus hayateae, Quercus spp. and Castanea spp. were analysed to better resolve the phylogenetic inference. Our results show that this cpDNA region harbour some informative sites that allow to discriminate all the different Fagus species. In particular, considering a short fragment of about 200 base pairs within the trnL intron, it is possible to distinguish the F. orientalis complex species (F. orientalis, F. taurica and F. moesiaca) from the remaining Fagus spp. (F. sylvatica, F. japonica, F. hayataea, F. crenata and F. grandifolia). This permits to analyse this fragment also in ancient samples, where DNA is usually highly degraded. Therefore, in order to elucidate the distribution of Fagus spp. present during the Last Interglacial period in the Italian Peninsula, we then sequenced this short cpDNA fragment in a sub-fossil sample, dated at least 45,000 years ago. The ancient DNA sequences demonstrate for the first time that, in contrast to current knowledge, the F. orientalis complex was present during the Tyrrhenian period in what is now the Venice lagoon (Italy). Moreover, we report a new method for direct amplification of ancient cpDNA from raw sediment samples, and we demonstrate its efficiency and reproducibility following the most stringent current standards for validation of ancient DNA sequences.

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KEYWORDS:
phylogeny and phylogeography; trnL-trnF cpDNA; ancient samples; Western Euroasiatic Fagus species; last interglacial period.

Nucleotide and haplotype diversity in candidate genes related to forest tree adaptation.

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The objectives of the project are to identify mutations of adaptive significance based on nucleotide diversity pattern analysis in sessile oak (Quercus petraea) and in maritime pine (Pinus pinaster). In total 35 gene-fragments were chosen for each species. These candidate genes are related to bud phenology and drought stress tolerance. The estimates of nucleotide diversities were moderately high with $\pi = 0.00589$ on average for oak, and $\pi = 0.00621$ on average for pine. For comparison, estimates of nucleotide diversity in the crop species analyzed so far range from high ($\pi = 0.0173$) in maize to low ($\pi = 0.0011$) in soybean. The few data available in forest trees (e.g. Pinus taeda, Poplulus sp) are similar to those obtained in this study. Together with the low linkage disequilibrium window frequently observed in these outbred, species, such a relatively high level of polymorphism constitutes a strong case toward the development of association mapping approaches based on targeted genes, to identify functional variation to a scale that could not be reached by classical QTL mapping experiments.

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KEYWORDS:
nucleotide diversity, candidate gene, SNPs, pine, oak

Glutamine synthetase as a marker of maritime pine populations in Morocco

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In Morocco, maritime pine (Pinus pinaster) is being degraded under the effect of an increased anthropic activity and unfavourable climatic conditions. In addition, the genetic sampling of this species in this region using molecular marker is generally poor and based on a few populations. Glutamine synthetase (GS, EC 6.3.1.2) is a crucial enzyme in ammonium assimilation in higher plants. Two different isoforms of glutamine synthetase named GS1a and GS1b have been identified in pine and their corresponding genes cloned and characterized (Cantón et al. 1993; Avila_Sáez et al. 2000). , we have for this study chosen GS1a and GS1b sequence tagged site (STS) markers followed by CAPS methods as an approach to analyse the genetic diversity of maritime pine in Morocco.

In general, the STS marker of the glutamine synthetase gene followed by CAPS has a low degree of polymorphism in the genomic region analysed. Further molecular analyses will be required to postulate whether or not, during the species history, the climatic changes favoured the selection of monomorphic or polymorphic GS genes. Moreover, our results provide new insights about a possible origin of the maritime pine in the north of Morocco. An assumption of this work is that the diversity measured may be a good representation of the capacity of the maritime pine species to be adapted to the environmental changes .

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KEYWORDS:
STS marker, Pinus pinaster, genetic diversity, CAPS, glutamine synthetase.

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I-P67

Analysis of gene expression during bud burst initiation in Norway spruce via ESTs from subtracted cDNA libraries

Igor A. Yakovlev[1],†, Carl-Gunar Fossdal[1], Øystein Johnsen[1], Olavi Junttila[2], Tore Skrøppa[1]

We have prepared four subtracted cDNA libraries, forward and reverse, representing genes predominantly expressed in a tree from early flushing (EF) and late flushing (LF) families of Norway spruce, during four weeks before bud burst (for both LF and EF) and seven weeks before bud burst for LF (sampled the same date). Two subtracted libraries during four weeks before bud burst for EF and seven weeks before bud burst for LF were analyzed using PCR-Select Differential Screening Kit (Clontech). And two subtracted libraries during four weeks before bud burst had been partially sequenced.

Differential screening reveals that in LF at seven weeks before the bud burst considerably upregulated senescence-associated genes, auxin-repressed proteins and several transcripts without similarities in the Database.

Partial sequencing is shown, that in the early library, ESTs encoding proteins of the photosynthetic apparatus, energy metabolism, stress (abiotic and biotic) and senescence related proteins were abundant. In the late library, ESTs encoding metallothionein-like and histone proteins as well as transcription factors were more abundant. We used quantitative RT–PCR to study the expression patterns of 25 chosen genes, and observed that the highest levels of activity for most genes were present when plants were still ecodormant. Late flushing is not a result of a simple delay in gene activity, but rather a consequence of an active transcriptional process. The putative role of the studied genes in regulation of bud burst timing is discussed. Among the candidate genes found, the most interesting ones were the DNA-binding factors, water-stress related genes and metallothioneins. Expression patterns of some genes involved in chemical modification of DNA and histones support our suspicion that epigenetic factors may be involved in the timing of bud burst. In the obtained transcriptomes, we were not able to find genes commonly recognized to be involved in dormancy and bud set regulation (PHY, CRY, ABI, etc.) in angiosperm plants.

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KEYWORDS:
Bud burst adaptive traits, contrasting-flushing trees, subtracted cDNA libraries, qRT–PCR, gene expression

COST

SESSION I

FUNCTIONAL GENOMICS OF TREE HEALTH

Using candidate genes for studying season-related responses in birch (*Betula pendula* Roth).

***Markku K.Aalto*, Stephen Rudd, Annikki Welling, Janne Laine, Pekka Heino and E. Tapio Palva.**

The silver birch (Betula pendula Roth.) is economically important tree species growing across boreal and temperate areas at Northern hemisphere. It is an extremely winter hardy perennial and we are using novel tools to unravel the molecular mechanisms leading to winter hardiness. We are interested in elucidating the molecular events taken place during development of hardiness at the end of growth season. For this, we are utilizing the information derived from a large-scale expressed sequence tag (EST) program which has provided a unigene set of 21 278 birch genes. A statistical comparison of the libraries used in EST sequencing revealed genes induced by different environmental cues. In addition, we have completed a thorough comparison between all available larger genomic and cDNA libraries throughout the plant kingdom.

Using the data from the EST program we have cloned genes that are predicted to be involved in hardiness development both at signaling and target gene level. We identified several putative CBFs of which two were chosen for functional studies. Low temperature stimulus induced the expression of birch CBF genes rapidly in growing birch leaves and in stems and buds of dormant, short day-grown plants. The expression was down regulated after few hours of low temperature stimulus. Interestingly, birch CBFs were also transiently expressed in response to freeze-thaw treatment but only during the thawing phase.

Looking for candidate genes in the detection of seasonal information (long vs. short day-signal) we cloned a birch gene (BpCOL2) encoding a member of a CONSTANS family. Transgenic expression in Arabidopsis and birch has shown that the gene is involved in root morphology.

Furthermore we have cloned a small family of related genes encoding a proteins belonging to a subgroup of dehydrins. These genes are seasonally regulated in opposite ways.

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Gene expression profiling of *Populus alba* in response to ultraviolet–B radiation

Giovanni Emiliani[1]**, Nathaniel Street**[2]**, Donatella Paffetti**[1]**, Raffaello Giannini**[1] **and Gail Taylor**[2]

Depletion of stratospheric ozone has led to an increase of ultraviolet-B radiation (UVB) on the surface of the earth; UVB radiation induces several physiological and morphological responses and is potentially deleterious to plant growth and development. The molecular mechanisms of UVB perception and UVB induced acclimation processes are still unclear. Here a microarray technology approach was used to asses UVB induced transcriptome change in poplar (Populus alba L.) under a realistic simulation of increased UVB radiation conducted in a controlled environment. The UVB radiation causes a dramatic change in gene expression, with more than 570 upregulated ESTs and 750 downregulated ESTs differentially expressed between irradiated and control plants after 30 hours of treatment. UVB radiation induced transcriptome change involved many pathways, with a strong down regulation of photosynthetic genes and the up regulation of the phenylpropanoid (lignin and flavonoid) and oxidative stress metabolism. Some pathways influenced by UVB radiation are shared with other biotic (Pathogenesis related proteins) and abiotic stresses (dehydration, heat shock, wounding); nevertheless UVB radiation also affected specific molecular targets such as DNA repair pathways and nucleic acid and protein turnover. This first microarray transcriptome analysis of poplar under UVB radiation highlights main pathways for future research.

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KEYWORD:
UVB radiation, poplar, microarray

A Genetical-genomics approach to understanding abiotic stress response in *Populus*

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Drought and ozone are both important abiotic stresses that severely impact forest productivity and both are likely to become increasingly prominent in Europe as a result of global warming and resultant climate change. Although much has been learnt about the control of ozone and drought response mechanisms in recent years, we aimed to identify functionally-significant genes involved in the control of abiotic stress responses and to examine how important transcriptional control may be in explaining species-specific response mechanisms.

We examined the response of an F_2 QTL mapping population to both chronic soil drying and ozone exposure. The grandparental responses were also examined and compared. The P. deltoides and P. trichocarpa grandparents were selected from relatively dry and wet regions of the USA respectively and we therefore hypothesised that natural variation in response to drought would segregate in the F_2 population. Our previous work had shown that the two species displayed highly contrasting responses to acute ozone exposure. We have also shown that the two species differ in their control of stomatal closure in response to abiotic stress.

The release of the Populus genome sequence enables alignment of the physical sequence with genetic maps of mapping populations: QTL were mapped for drought and ozone response in order to identify genomic regions of functional significance. We then identified a set of genotypes at either end of the population extreme for each stress response and compared the transcriptional response of these high- and low-responsive genotypes to identify transcripts of functional importance. The overlap of identified transcripts with QTL regions was examined.

Marked transgressive segregation was observed in response to drought and ozone stress within the F_2 population, including for leaf pigment content, expansion, abscission, and necrosis. It was found that the transcriptional responses of the high- and low-responses genotypes were highly divergent, suggesting that gene expression control play a significant role in explaining species-level responses.

Such an approach may prove invaluable as a means of selecting functional candidate genes for subsequent expression QTL mapping or for examination within a linkage disequilibrium population for fine mapping.

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KEYWORDS:
drought stress; ozone stress; QTL mapping; transcriptomics; genetical genomics

Leaf development and senescence in elevated CO2: linking transcriptomics and QTL analysis

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Atmospheric CO_2 concentration has risen approximately 39 % following the industrial revolution and continues to rise at an increasing rate. Future projections identify an atmospheric CO_2 concentration of 550 ppm by the year 2050, a 96 % increase on pre-industrial levels. Independent of the associated global warming, this increase in CO_2 is known to be having profound effects upon terrestrial vegetation. It is likely that this change may be acting as a selection pressure on plants resulting in adaptive responses to rising CO_2.

The changing physiology of forest trees to elevated CO_2 are well documented, but little research has been conducted to understand genetic and genomic responses. Here we address this using cDNA microarray technology and QTL analysis. For the transcriptome study poplar trees were grown using Free-Air Carbon Dioxide (CO_2) enrichment (FACE) exposure at the EUROFACE site (www.unitus.it/euroface). This facility is a 9 hectare plantation of poplar trees growing in central Italy, where experimental plots enrich the atmospheric CO_2 concentration to 550 ppm. The aim of the experiment is to quantify carbon uptake, sequestration and loss by this potential biomass crop in a future high CO_2 world. During the study reported here, the plantation was characterised by a closed canopy following six years of CO_2 enrichment.

During the growing season of 2004, young and semi-mature leaves of P. x euramericana were sampled from three time points, to identify transcripts sensitive to CO_2 enrichment as the leaves developed. Surprisingly, both the POP 2 (www.upsc.se/microarray) and PICME (www.picme.at) cDNA microarrays confirmed that few transcripts were differently expressed. However, the results indicated that development stage of the leaf had a strong influence upon response to CO_2 treatment. Mature leaves were also sampled around the time of bud-set and late into the autumn to identify transcripts responsible for an observed delayed senescence in elevated CO_2. Late in senescence, transcripts involved in the anthocyanin and raffinose pathways, which are known to provide stress tolerance, were up-regulated in elevated CO_2. During the course of senescence, of those ESTs significantly differentially expressed between treatments, 293 unique to ambient CO_2 were two fold down-regulated where as none of these transcripts were two fold down-regulated in elevated CO_2.

These studies have given a first insight into the genetic basis of poplar response after long-term adaptation to elevated CO_2. Candidate genes identified by the microarrays, and QTL identified from a poplar mapping population exposed to elevated CO_2, are under further investigation. Further work using poplar exposed to elevated CO_2 in controlled environments is underway. Here we aim to link both transcriptomic and proteomic approaches.

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KEYWORDS:
Elevated CO_2, Microarray, Poplar, Development, Senescence.

Involvement of a copper chaperone (CCH) in the response of poplar to cadmium

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Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress. These mechanisms include exclusion, chelation, and compartmentalization of metal ions. Unlike in annual plants, very little is known about these mechanisms in forest trees. We used microarrays to elucidate the molecular response of poplar to cadmium. We showed that a gene encoding a copper chaperone (CCH) is up-regulated in hydroponically cultivated poplar clones treated with cadmium. The encoded protein is 78% identical to the Arabidopsis CCH which has been shown to complement ATX1 of yeast (Himelblau et al. 1998). ATX1 is a small copper binding protein which participates in copper trafficking and in the defense against oxidative stress. To study the role of CCH in response to cadmium, we analyzed Arabidopsis plants lacking the CCH gene. Compared to wildtype plants these mutants showed increased sensitivity to cadmium, indicating that CCH is part of a tolerance mechanism to cadmium. Physiological roles of the copper transporter will be discussed.

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KEYWORDS:
heavy metals, cadmium, poplar, microarrays, copper chaperone

Association mapping of resistance to rust disease in poplar

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The foliar rust caused by the fungus Melampsora larici-populina Kleb. is the most devastating disease affecting the European poplar plantations. We have identified a factor of quantitative resistance, Rus, inherited from the genotype Populus trichocarpa 101-74, which can reduce by 60% the level of infection in laboratory and which has a significant effect in field. This factor is localized on the genetic map of P. trichocarpa 101-74 based on the analysis of one F1 interspecific family P. deltoides x P. trichocarpa. Map based cloning of Rus is under development, fine mapping and construction of BAC contig are underway. The alignments with the sequence of P. trichocarpa Nisqually-1 makes it possible to target the area of interest. We defined an exploratory zone of approximately 400 kb on which we already detected 28 genes. Ten of them are re-annotated as Resistance Gene Analogs (RGAs). Predicted genes have been investigated as candidate genes for fine mapping and association study. Association between Rus and those candidate genes has been first investigated in a collection of 94 individuals of P. trichocarpa. The collection was phenotyped for rust resistance under control conditions and in the field. The individuals have been genotyped by EcoTILLING.

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SESSION II

FUNCTIONAL GENOMICS OF TREE MATURATION AND REPRODUCTION

Genetic approaches to alter tree growth

Matthias Fladung*, Hans Hoenicka, Siegbert Melzer

A number of gene technological approaches using hormonal and flowering genes have been followed in the past to alter tree growth and development. Hormones are involved in regulation of many features during plant development like shoot growth and flower formation. Classically, modifications of shoot growth resulting in so-called dwarf or semi-dwarf mutants are very often caused by mutations in genes encoding proteins that regulate synthesis and/or signalling of gibberellin, a major plant hormone. The genetic transfer of genes altering hormonal balance, e.g. rol genes, ipt and GA20oxidase, for changing shoot growth, may be an attractive starting point when aiming for modifying plant phenotypic and wood anatomical features in trees.

Although many natural or induced mutant trees are available, the nature of the mutations behind them remain broadly unknown. Out of these, unfortunately, only a few number of flowering mutants are known for forest tree species. One example is the Picea abies ‘acrocona’ mutant, which is characterized by precocious flowering and terminal cones. However, the molecular basis of this mutation remains also unknown.

The use of genes involved in different developmental processes, e.g. flowering or flowering time control, can represent an alternative for studying tree growth development. On the other hand, surprising results are sometimes obtained. The meristem identity gene leafy (LFY), for instance, induces precocious flowering in transgenic poplar. Additional side effects of this gene are dwarfism, single flowers (instead of catkins) and the induction of gender change only in female clones. The Flowering Locus T (FT) induces also early flowering in poplar. Poplar plants transformed with FT show a more normal phenotype than those transformed with the LFY gene, but with slightly pending leaves. The homeobox gene BpMADS4 induces early flowering in birch and apple but not in poplar. However, this gene induces an “evergreen” poplar comparable to the oak turneri hybrid. Overexpression experiments using the flowering time genes SOC, FUL and FPF from Arabidopsis thaliana are ongoing in poplar. Their role in the induction of flowering and controlling flowering time in poplar will be assessed.

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KEYWORDS:
flowering time control gene; altering hormonal balance; poplar; tree development

Leaf determinants of productivity in poplar depend on environmental conditions and genetic background

***Sophie Y. Dillen,* Nicolas Marron and Reinhart Ceulemans**

Biomass production is a complex trait, which could be partitioned into functional and structural components. The analysis of productivity components and their genetic control in Populus is a crucial step in the early selection process of highly productive genotypes: the breeder will use predictor traits showing the highest genetic variability and the tightest correlation with biomass production. Genotype x environment (GxE) interactions are also an important factor to be considered because they might alter the relative performance of genotypes across environments, they can affect the relationships between productivity and its potential predictors, and they have the potential to influence the nature and magnitude of the selection response. In this context, two Populus families growing at two sites in Europe (i.e., northern Italy vs. central France) were used to investigate (1) the relationships between various leaf structural and growth traits and biomass production, (2) the dependence of these relationships on environmental conditions and genetic background (i.e., P. deltoides x P. nigra family vs. P. deltoides x P. trichocarpa family), and (3) the subsequent relevance of the use of these morpho-physiological traits as indirect indicators of productivity. Tree growth, leaf characteristics as well as the links between both were intensively studied during three weeks. The traits significantly differed between the two poplar families and were clearly dependent on site conditions. Three categories of leaf traits could be considered: (i) traits linked to whole tree growth irrespective of site and family (e.g., leaf area, petiole dimensions), (ii) traits for which the relationships with tree growth were dependent on site and/or on family (e.g., specific leaf area, nitrogen content, leaf number increment), and (iii) traits showing no links with tree growth irrespective of site and family (e.g., chlorophyll and carbon contents).

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A ring test for identity and distinctness in forest tree seedlots

***Berthold Heinze*, Wolfgang Arthofer, Barbara Fussi, and ring test participants**

Molecular genetics may offer attractive tools for the certification of forest tree seed moving in international trade. Any assays for that purpose must be selective, so that items that should be distinguished are actually discernible as different in the lab, and they must be robust and repeatable in different laboratory settings.

We have conducted a ring test among several European laboratories with seedlots of the ash tree, Fraxinus excelsior. Seeds from two seedlots were chosen and divided into three bags, so that two bags had seeds from an identical seedlot, labeled in a neutral way (A, B, C) and sent out to participating labs. A limited number of nuclear microsatellites are available in this species. Each lab independently prepared DNA from the seeds, analysed microsatellites, and assessed the question of which two “bags” are actually identical.

The results are very encouraging - each participant correctly identified the two identical “bags”. We will present an in-depth analysis on how compatible results from the different labs are when all details (allele sizes, frequencies, and calculated genetic parameters) are compared. We will discuss the possible role of such tests in law enforcement and quality control in this sector.

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KEYWORDS:
forest seed certification, microsatellites, quality control, multi-laboratory ring test

Winter dormancy, cold acclimation and circadian clock in chestnut

I. Allona, **C. Ibañez, R. Casado, A. Ramos and C. Aragoncillo**

Winter dormancy is a survival strategy that enables plants to persist periods of stressful environmental conditions. This process determines to what degree fruit crops will survive the winter and early spring without shoot and flower bud damage and, in long-lived forest species, the length of dormancy limits the growing season and thus affects wood production and quality. For these reasons, a thorough understanding of the molecular physiology of dormancy will be helpful to develop better approaches for plant genetic improvement. In our laboratory we have described that the circadian clock of chestnut undergoes significant disruption during winter dormancy and cold temperatures. The circadian clock is involved in the coordination and proper functioning of major metabolic pathways, and it controls different physiological processes. Therefore, the clock's disruption observed in chestnut during dormancy could control physiological key changes that take place in woody perennials during winter. In Arabidopsis, a number of circadian clock-associated protein components have recently been identified. TOC1, one of the main clock components, is a member of a small family of pseudo response regulators. Some of these members, have been proposed to provide a flexible output mechanism that can regulate a gene at any desired phase from dawn to dusk. Here we present the effects of low temperatures on the expression of several members of these gene family.

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Epigenetics, The Role In Plant Differentiation And Development.

Berdasco, M.(1,2); Fraga, M.F.(2), Cañal, M.J.(1,3), Esteller, M.(2) & Rodríguez, R.(1,3)

Cell differentiation and development are controlled through the temporal and spatial activation and silencing of specific genes. In contrast with animal development, plant development is plastic and strongly influenced by biotic and abiotic factors and require specific interactions between developmental programs and signaling pathways that must be coordinated at the level of chromatin organization. DNA methylation is the best-known epigenetic modification during ontogenic development but it is only a part of a complex molecular machinery that, in collaboration with histone modifications, constitutes a specific language (“epigenetic code”) controlling gene expression and chromatin structure. Our previously investigations were centered in the quantification and tissular distribution of DNA methylation and H4 acetylation variations affecting global chromatin, and their correlation with phase-change development and the gain of morphogenic competence arising from reinvigoration. These analysis allow to propose that global acetylated histone H4 and 5-methylcytosine amounts are specific indicators of the radiata pine phase change, and these maturation-related epigenetic variations could be imposed, at least partly, through specific states of tissue differentiation. But what happens at gene specific level?. In most cases, we do not know what genes are responsible of a determinate developmental state, and even less the up or down regulation of these genes. The role of DNA methylation in the control of specific genes related to plant differentiation and development were carried out by microarray technology and bisulfite sequencing. We found that hypermethylated promoters of specific genes are correlated with dedifferentiated cells and uncontrolled growth, while promoters from differentiated tissues with controlled proliferation were hypomethylated. We also confirmed that hypermethylation is associated to gene repression, being expression reactivated after treatments with demethylation drugs. Chromatin immunoprecipitation (ChIP) analysis confirmed that promoter hypermethylation and gene repression are associated to repressive modifications of the adyacent histones. Functional assays, by gene transformation, confirmed their implication in proliferation and plant differentiation.

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KEYWORDS:
DNA methylation, histone acetylation, cell proliferation, tissue differentiation, plant development

Differences and common features of developing somatic and zygotic embryos of oak (*Quercus robur L.*)

E. Wilhelm[1], C. Prewein[1], V. Sunderlikova[2], J. Salaj[2];

In several broad-leafed tree species, including also oak, somatic embryo (SE) maturation represents a major barrier to the commercialization of this vegetative propagation system. To improve the quality and germination frequency of SEs, comparative biochemical and molecular studies were performed to study the physiological changes during zygotic and SE development. The water status, proline, starch, endogenous ABA and IAA levels of the developing embryos were monitored in relation to morphological characters. The temporal and tissue specific expression patterns of the legumin gene, the major storage protein of oak, and several oak lea genes (late embryogenesis abundant proteins) were investigated. Differential expression patterns of some lea genes were found, whereas the expression in zygotic and somatic embryos of other lea genes is embryo specific and developmentally regulated. Variations in phytohormone levels in combination with changes in tissue water status seem to be important factors for both zygotic and somatic embryo development.

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Molecular approaches to understand regulation of embryo development in maritime pine (*Pinus pinaster*)

Sónia Gonçalves[1], Marta Simões[1], Margarida Rocheta[1], Margarida Oliveira[1,2], John Cairney[3], *Célia Miguel*[1]

Embryogenesis is a complex process that requires regulation of genes within the embryo proper and neighbouring seed tissues surrounding the embryo. Many genes are expressed during this process in a highly coordinated manner to ensure that the zygote develops into an organised multicellular structure. Angiosperm and gymnosperm plants evolved different embryo development pathways showing notable differences during seed development. In gymnosperms still little is known about the mechanisms involved in embryo development.

We describe sequence characterization and expression patterns (transcript quantification and in situ localization) of genes previously identified as being differentially expressed during pine embryogenesis. Additionally, expression data along embryo development of PpRT1, a gypsy-like retrotransposon previously identified and characterized in the genome of P. pinaster was obtained.

The PpRab1 gene putatively codes for a Rab GTPase, known to be involved in vesicular transport. The pattern of expression presented by PpRab1 both in maritime pine zygotic and somatic embryogenesis reveals that this gene is mainly involved in the early-to-middle stages of embryo development. The PpFBK1 transcript, also studied, codes for an F-box protein containing Kelch repeats. The combination of F-box proteins and the Kelch motif seems to be unique to plants and our results suggest that PpFBK1 may be involved in a process that is of particular significance in maturing embryos. Functional analysis of these genes is underway. Following previous characterization of PpRT1, which is a 5966 bp long gypsy-like retrotransposon, identified in the genome of P. pinaster, evidence of its transcription was found in early stages of pine zygotic embryo development and during embryo germination.

These studies may provide a basis for the identification of stage-specific markers useful for monitoring somatic embryo development and clues to overcome developmental constraints.

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embryo development, gymnosperm, gene expression

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Adventitious Rooting Genes In Distantly-Related Forest Species

Conchi Sánchez[1], Jesús M.Vielba[1], Enrique Ferro[1], Guillermo Covelo[2], Dolores Abarca[3], Belén S. de Mier[3], Alicia Solé[3], Carmen Diaz-Sala[3]

Adventitious rooting is a postembryonic organogenic process in which root meristems are organized at positions where roots are not originated in normal development. The ability to form adventitious roots from stem cuttings is a key step for propagation of selected adult trees. However, in many forest species, the regeneration capacity of adult cells is limited, especially at maturation, an age-related developmental process that also affects morphology and growth rate. In an attempt to obtain information from genes expressed during adventitious root formation, we have identified expressed sequenced tags (ESTs) associated to adventitious rooting by sequencing cDNA suppression subtractive hybridization (SSH) libraries from rooting-competent cuttings under induction conditions of two distantly-related forest species, Pinus radiata and Castanea sativa. Among them, the identification of two SCARECROW-LIKE (PrSCL1 and CsSCL1) genes from pine and chestnut respectively, which are auxin-induced in rooting-competent cuttings during early stages of adventitious root formation, has been carried out. Functional analysis using Arabidopsis thaliana de-rooted plants shows that the auxin-responsive SCARECROW gene is required for adventitious root formation in cuttings at different stages of plant development.

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KEY WORDS:
adventitious rooting, SCARECROW, maturation, pine, chestnut

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SESSION III

FUNCTIONAL GENOMICS OF WOOD FORMATION

Hormones and wood formation

Sundberg, Björn

Wood development canibe much modified by experimental manipulation of the balance of auxin, gibberellins and ethylene in the wood forming tissues. These observations have led to several long lasting hypotheses about hormonal control of wood variations in the tree stems. We have evaluated some of these ideas by analyzing the endogenous balance of auxin and GAs in developing wood of Populus under experimental and intact conditions. We have also used microarray analysis to look at global gene expression in the same tissues under different hormonal exposures. Whereas polarly transported auxin is required for cambial cell division and xylem differentiation and induce many thousands of the poplar genes, (including GA biosynthesis genes), GAs have a more specific role in primary walled dividing and expanding cells, and also induce a much smaller set of genes. Interestingly, we found evidence for GA induced auxin transport that may explain part of the synergistic effects observed between GAs and auxin in cambial growth. We have also constructed ethylene insensitive Populus trees by transgene expression of the mutated AtETR1 gene to explore the role of this hormone in wood formation. With these trees we have confirmed that many of the earlier observed ethylene effects are transduced through the ETR1 receptor, and we have explored the downstream genes that are induced by ethylene. We provide evidence that ethylene is involved in quantitative aspects of the tension wood response.

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Analysis of gene expression in cambial zone of poplar (*P. alba*) in response to water deficit

Berta Monica, Caparrini Simona, Giovannelli Alessio*, Sergio Rossi **, Sebastiani Federico*, *Milvia Luisa Racchi***

Poplars are among the fastest growing trees in temperate latitudes. Their high productivity is associated with large water requirements. As a consequence water deficit condition is the major limitation to growth and represents the main problem affecting wood productivity. Wood formation is being increasingly studied at cellular and biochemical levels; however, gene expression and regulation during wood formation in response to abiotic stress are poorly understood. We used physiological and morphological parameters coupled with DNA microarrays to study the effect of reduced water resources on cambial activity and developing xylem cells.

Water stress was induced on poplar cuttings (P. alba, Marte) by withholding water, while irrigated trees were used as a control. Recorded variables include stem traits (stem length increase, radial growth) leaf structural (total leaf area, leaf area increase, and leaves number) and functional traits (predawn leaf water potential (_pd) stomatal conductance (g) and CO_2 assimilation (A)). After 9 days of totally withdrawing water, leaf _pd fell down to -0,8 MPa resulting in a significant reduction of the stomatal conductance, CO_2 assimilation, consistent increment of stem shrinkage and suspension of the radial growth. At this time samples were taken for histological analysis and RNA extraction from non-irrigated and control plants. Microscopic observations showed that the width of cell-expansion zones differed between stressed and control cuttings. Our results showed that, under these conditions, poplar cuttings put into action mechanisms to reduce water loss such as stomatal closure, water translocation from the stem to the leaves and primary meristems. In fact, no significant differences were recorded between treatments in term of leaf RWC, total leaves number, total leaf area increase and stem length increase. For microarray analysis, RNA was extracted from the cambial zone of lyophilised stems. Transcript analysis was performed using PICME chips based on a set of 27709 clones assembled from poplar EST collections of various tissues. Genes that consistently displayed higher or lower transcript levels were grouped into functional categories. The majority of the genes with altered transcription levels belong to few functional categories such as stress response, signal transduction and transcription, protein turnover and mobilization of carbohydrates. The relevance of the metabolic changes at cambial zone level in relation to plant growth during water stress is discussed.

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KEYWORDS:
cambium, water stress, gene expression, microarray, poplar

High-resolution transcript profiling across tension wood developmental zones

Kumar M[1], Ellis B[2], Sundberg B[1]

In response to a gravitational stimulus, woody plants produce a specialised form of wood known as reaction wood (tension wood, in hardwood species). A dramatic feature of tension wood fibres is formation of a so-called G (gelatinous) layer which is formed as an inner cell wall, replacing part of the S2 layer. The G-layer is composed of almost pure crystalline cellulose, which implies that biosynthesis and deposition of lignin and hemicellulose is suppressed when G-layer formation is induced. This developmental switch thus provides an excellent system in which to identify genes and proteins involved in cell wall biosynthesis. We have performed high-resolution gene profiling across the tension-wood forming zone in Populus, using 30 μm tangential sections obtained with a cryo-microtome. A small number (3 to 9) of sections originating from specific zones along the developmental gradient associated with secondary cell wall biosynthesis in tension wood trees were pooled together, making a total of 6 pools that span this gradient. From upright trees forming normal wood, 3 sample pools were obtained that covered the same developmental space. To provide biological replication, two different tension wood trees and two normal wood trees were sampled independently. Total RNA extracted from the pooled sections was amplified using an in vitro transcription-based technique to generate sufficient amounts of RNA/cDNA for microarray hybridisations. Three technical replicate hybridisations were performed for each sample (including dye-swaps), using the 26K POP2 cDNA microarray. The resulting gene expression profiles were evaluated using both supervised and unsupervised clustering. Among the genes whose expression was found to respond strongly across the various tension wood developmental zones, and at the same time are differentially expressed compared to their behaviour in normal wood, are many of the homologues of FLA12, ACC oxidase, COBRA and cellulose synthase.

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In situ genetic variability estimation of wood, phenology and morphological traits in a natural stand of *Quercus.*

L. Sanchez[1]; S. Gerber[2]; J. C. Vilardi[1,4]; A. Ducousso[2]; J. L. Puech[3]; C. Rathgeber[5]; P. Rozenberg[1]

With non domesticated species, like most forest trees, the assessment of heritability for traits of adaptive relevance represents one of the first steps granting future conservation and breeding programs. This essential step, however, is lengthy to conduct on long-lived species, implying mostly ex situ experiments and little environmental resemblance to the original populations. A major obstacle for in situ genetic studies has been the difficulty in grasping the extent of family interrelations in natural populations. Recent developments in molecular biology, however, are making genetic markers readily available and more efficient for measurement of relatedness. Theoretical improvements have also been proposed to infer the heritability and the evolution of genetic variance from the joint analysis of natural relatedness and the in situ quantitative trait variation. Here we propose one of the first evaluations with forest tree species of this in situ approach. The studied population comprised 280 trees of two interbreeding species: Quercus petraea and Quercus robur. This study considered as many as 60 different traits: foliar morphology, growth, phenology, wood properties, wood defects and wood biochemical compounds, some of which with no published records of heritability estimations. A total of 17 highly polymorphic microsatellite markers were studied in the sample. The genetic markers revealed low levels of relatedness and variance of relatedness, which are in principle unfavourable conditions for the detection of heritability. We proceeded by ranking the estimates among traits, and carried out randomization test to setup confidence intervals. We found that those traits known to be highly heritable in the species or at least in related taxons (i.e. phenology, wood physical and biochemical properties) appeared at the top of the trait ranking for the two species, with highly significant associations between the genetic distance revealed by the genetic markers and the phenotypic resemblance. On the opposite, other traits known to have moderate heritabilities, like growth, showed positive but no significant associations. Moreover, the two species exhibited different heritability patterns for leaf morphology traits, known to discriminate Quercus species. Although this in situ methodology cannot substitute its more precise ex situ counterpart, it could become an excellent exploratory tool of the natural occurring variation, and the detection of valuable populations for conservation purposes. We discuss the role of some useful add-ons that might further improve this in situ approach, like spatial statistics and simulation studies.

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KEYWORDS:
Heritability, natural population, microsatellites, relatedness, genetic variation

Molecular and phenotypic characterisation of wood forming tissues along a cambial-age gradient in maritime pine

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Environmental, developmental and genetic factors affect the variation of wood properties at the chemical, anatomical and physical levels. In this study, we explored the phenotypic plasticity observed along a cambial age gradient to test the hypothesis that the variation in wood characteristics could be due to the differential expression of genes during wood formation. Differentiating xylem samples of maritime pine (P. pinaster Ait.) were collected from the top (juvenile wood, JW) to the base (mature wood, MW) of an adult tree. These samples were first characterized by FTIR and analytical pyrolysis. Two main groups of samples, corresponding to JW and MW associated samples, could be distinguished based on cell-wall chemical composition. A genomic approach combining large-scale production of ESTs, gene expression profiling (reverse Northern using high-density-filters) and quantitative proteomics analysis (2DE-MS/MS), allowed to identify differentially expressed genes/proteins along this gradient. Of a total of 3,512 studied genes, the expression of 262 (7.5%) presented a strong and significant cambial age effect. These genes were clustered by their transcriptomic profiles into four distinct groups; the large ones corresponding to genes over-expressed either in the JW (35.5% of the genes detected), or MW (27.1% of the genes) forming tissues, respectively. The proteome reference map of differentiating xylem consisted of 1,372 spots. A total of 231 spots showed significant variation along the cambial age gradient, among which 66% preferentially accumulated in MW forming tissues.

The two main results of this experiment are: i/ a good agreement (in terms of functional categories) between the levels of gene expression revealed by the transcriptomic and proteomic analysis of the same wood forming samples, and ii/ a good fit between the molecular mechanisms and the phenotypic differences found between JW and MW.

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Unravelling the vascular cambium in Poplar and Arabidopsis

Brian Jones

As the vascular cambium is the ultimate source of the majority of forest-derived biomass, it is increasingly imperative that we understand how it functions. We are taking a molecular genetic approach to investigate its structure and function. In mammalian systems, many of the genes important for the function of one stem cell niche are critical in the functioning of at least one other. Several apical meristem regulator homologues that have been shown to be differentially expressed across the vascular cambium are being used to identify components of the cambium through a reverse genetics approach. To date we have concentrated on members of the GRAS family of transcription factors. Partial suppression (70-80% reduction in steady-state mRNA levels) of one member of this diverse and extensive gene family gene leads to an almost 40% average increase in biomass production over WT trees after 2 _ months in the glasshouse. This phenotype appears to be due to an overall acceleration in both primary and secondary growth rather than an increase in any one component of growth. We are investigating the basis of this phenotype through a combinatorial approach in Poplar and Arabidopsis. The ultimate aim of this and other investigations we are pursuing is to deconstruct and understand how the still largely uncharacterised vascular cambium stem cell niche functions.

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First insight into micro-genomics of cambial activity per cell type in poplar.

Nadia Goué[1], Björn Sundberg[2], Ewa Mellerowicz[2], Marie-Claude Lesage[1] and Philippe Label[1]

As a model tree, poplar is increasingly studied at the genomic level. Going deeper into the understanding of relationships between cambial activity and wood formation, we have developed in collaboration between INRA and UPSC a method to analyze gene expression at the cellular level in the poplar cambium. After general considerations explaining why and how to perform microsampling at the cell level, we will develop some results obtained during the PhD work of Nadia Goué showing main gene families expressed in the fusiform and radial cells microdissected from the poplar cambium. These results will be also analyzed in line with the poplar genome sequence obtained recently

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CLOSING LECTURE

The Forest-Based Sector Techonology Platform in the Context of European Forest-related research

Yves Birot

The history of scientific cooperation in Europe dates from the 1950s to the198 80s is characterized by the development of common large-scale facilities and cooperative networks (COST, ESF, EUREKA) through multi-country agreements. During this phase, the European Commission established the Joint Research Centre. The momentum of EU Research Policy was initiated in 1984 with the launch of the Framework Programme for Research and Development. This policy was highly successful with the emergence of a European Scientific Community. A new momentum was given in 2000 with the European Research Area and FP6, and with the commitment in 2002 of EU member countries to allocate 3% of their GDP to research.

FP7 has opened new avenues, in particular with the development of **European Technology Platforms,** whose aim is to involve all stakeholders of a given industrial sector, to make it through scientific and technological research, more knowledge based, more customer focused and more innovation oriented for a better competitiveness. The paper describes the development of the European **Forest-based Sector Technology Platform** (FTP), This unprecedented initiative has resulted in common long term (2030) vision and objectives, and in a ambitious **Strategic Research Agenda** (SRA), whose structure and content in terms of priority Research Areas are briefly presented.

The implementation of the SRA into Research Programme and Projects will require using all available instruments, such as those provided by the EC through FP7, but also through national and multinational programmes, and existing bodies such as EUREKA and **COST**. Increased funding for research from industry, EU and national public sources is a major goal.

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POSTER

Evaluation of PCR-RFLP for forest seed certification by a multicenter ring test study

Wolfgang Arthofer[1]**, Barbara Fussi**[1] **and Berthold Heinze**[1]

PCR-RFLP is a simple and straightforward technique for mutation detection in PCR products with unknown sequence. Here we describe the results of a ring test for evaluation of the reproducibility of the method in different laboratories. This was done with a view to evaluate approaches for a genetic certification of origin for forest tree seeds. At the Federal Research Center (BFW), DNA samples of 24 ash (Fraxinus excelsior) trees were shipped together with cpDNA primers to six centers for subsequent PCR amplification and restriction analysis. Results were returned to the BFW as haplotype tables and gel photos.

719 out of possible 1008 restriction patterns (24 samples * 7 primer combinations * 6 centers) could be evaluated. Missing results were mainly caused by insufficient yield of PCR product. 682 observed patterns (94.85 %) were identical with those retrieved at the BFW. In depth analysis of aberrant patterns revealed misinterpretation due to incomplete digestion of the amplicons as the main source of error.

While PCR-RFLP provides fast results and requires only standard instrumentation available in most genetic laboratories, emphasis should be put (a.) on optimization of the PCR amplification itself to yield sufficient and single-banded product and (b.) on quality control of the digestion step including standard DNA with known restriction patterns. Once these issues are adequately addressed, it seems possible to determine chloroplast haplotypes and hence, regions of origin, for certain forest trees across many laboratories.

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KEYWORDS:
PCR-RFLP, forest seed certification, cpDNA, quality control

Functional characterization of *PtaRHE1*, an aspen RING-H2 gene expressed in vascular tissues

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Vascular secondary growth results from the activity of the vascular cambium, witch produces secondary phloem and secondary xylem. By means of cDNA-AFLP analysis along aspen stems, several potential regulatory genes involved in the progressive transition from primary to secondary growth were identified (van Raemdonck et al., 2005). One of these genes, PtaRHE1 encoding a RING-H2 finger protein, was found to be expressed in the ray initials and their derivatives within the cambial zone. Several RING-finger proteins have been shown to have an E3 ligase activity in the ubiquitin mediated targeted proteolysis (Stone et al., 2005). Promoter-driven GUS expression was studied for ATL2, the closest A. thaliana homolog to PtaRHE1. In mature plants, a strong GUS signal was detected in the root hypocotyl junction where secondary growth was shown to take place. These results indicate a potential role of this gene in vascular tissue development and/or functioning.

To further characterize PtaRHE1, transgenic tobacco lines overexpressing this gene have been established. These transgenic lines are strongly affected in their growth and development and exhibit reduced stem growth and a curled leaf phenotype, suggesting an alteration in various developmental processes. Experiments are now planned to characterize these transgenic lines at the biochemical level by the analysis of lignin content and composition and the identification of potential target to PtaRHE1.

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KEYWORDS:

cambium, transition to secondary growth, poplar, RING-H2

REFERENCE:

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Developing genetic linkage maps of *Pinus pinaster* using SAMPL, SSR and cDNA-based markers.

Nuria de María[1], Candela Cuesta[2], Raúl Fernández[1], Luís Díaz[1], M. Teresa Cervera[1]

Pinus pinaster, the most abundant autochthonous conifer of the Iberian Peninsula, shows high levels of genetic diversity, not only between provenances but also between populations and even between individuals from a same population. This broad variability embeds a great potential for adaptation to changing environmental conditions.

The goal of our work is the study of the response capacity of Maritime pine (Pinus pinaster) to changing environmental conditions. One of the approaches that we are following is the development of linkage maps from a population of the central region of Spain that suffers seasonal drought (location name Coca). These genetic maps will enable the study of associations between markers and adaptive and productive traits. We are using high throughput markers such as SAMPLs, as well as co-dominant markers such as SSRs and cDNA-based markers, to analyze a full sib F1 progeny that segregates for several adaptive and productive traits. The use of a common set of anchor markers will allow the identification of homologous linkage groups when compared with already published maps not only from Maritime pine (Pinus pinaster) from Landes and Corsica, but also from other conifer species. Comparison with other Pinaceae will allow to acquire wider knowledge about genome structure as well as exchange information among mapping pedigrees.

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KEYWORDS:
adaptation; genetic map; Pinus pinaster.

Sequencing and annotation of large genomic regions in Norway spruce (*Picea abies* (L.) Karst)

Emanuele De Paoli[1], **Michele Morgante**[1]

Conifers have large and complex genomes due to a high content of repetitive DNA. Transposable elements are known to play a major role in the genome structure and evolutionary dynamics of conifer species. Nevertheless, to date the study of transposable elements in gymnosperms has been largely based on degenerate PCR amplification of conserved domains and the exhaustive characterization of full-length elements is far from complete owing to the scarcity of long range genomic sequences. We report the annotation of four large regions randomly selected from the genome of Norway spruce (Picea abies) and accounting for a total of ~400 Kb. This survey contributes significantly to enlarge the retroelement repertory currently available for genomic analysis of conifer species by the identification of novel families and complete retroelements, including the first non-autonomous LTR-retrotransposons ever reported in conifers. Moreover, we provide the first insight into the local organization of a complex conifer genome showing nests of retrotransposons inserted into each other as commonly found in middle-to-large genome angiosperms. Estimates of retroelement insertion ages reveal an old ancestry dating back to the differentiation of Pinaceae species and insertions of related retrotransposons suggest that different waves of retroelement mobilization may have occurred during the evolution of conifers. The survey of large genomic regions with respect to the content, physical arrangement and age of retroelements may provide a substantial contribution to our understanding of genome organization and evolution in conifers. Finally, the characterization of full-length transposable elements is an essential step required for the satisfactory annotation of genomic libraries from newly investigated conifer species.

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KEYWORDS:
conifers, genome organization, retro-elements.

Arabidopsis as a tool to study the molecular basis of maturation-related decrease in adventitious root formation in forest species

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Adventitious rooting is a developmentally controlled process that limits the success of clonal propagation programs in forest species. The development of molecular tools to characterize the process is difficult in most forest species due to the limited knowledge of their genome, the difficulty to transform these species and the time required to evaluate the phenotype of transgenic plants. This limits the possibility of identifying putative regulatory genes and testing their function in adventitious root formation. In this work, we describe the potential of arabidopsis as a tool to complement molecular studies of adventitious root formation in forest species. We have developed an experimental system using arabidopsis hypocotyls that reproduces the developmental control and the auxin requirement that occurs in forest species such as pine and chestnut. Using this system, we show that adventitious rooting in the hypocotyl of arabidopsis decreases with plant age and is partially related to plant maturation. In addition, we have identified genes that are specifically expressed in roots and in rooting competent pine and chestnut tissues under inductive conditions, and are also specifically expressed in the arabidopsis root meristem. This suggests the possibility of conserved mechanisms in the determination of root meristems among distantly related species, and opens the possibility of carrying out complementation analyses in arabidopsis mutants with pine and chestnut candidate genes. With this purpose, we are carrying out functional analyses of adventitious rooting in arabidopsis mutants. Preliminary results show that we can measure quantitative differences in adventitious rooting using genotypes with mutations in genes that are expressed in arabidopsis root meristems.

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KEYWORDS:
Adventitious rooting, regulatory genes, maturation, Arabidopsis

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Isolation of a *SHORT-ROOT* gene from *Pinus radiata* and its expression during adventitious root formation

Alicia Solé[1], Conchi Sánchez[2], Jesús M. Vielba[2], *Carmen Díaz-Sala*[1]

SHORT-ROOT (SHR) is a putative transcription factor of the GRAS family which has been involved in the establishment of root meristem cell identity and root patterning in Arabidopsis. In an attempt to study if this gene is required for rooting in forest species, we have cloned a SHR gene (PrSHR) from roots and rooting-competent cuttings under inductive conditions of Pinus radiata. The predicted protein sequence contains domains characteristic of the GRAS protein family, and shows a strong similarity to the Arabidopsis SHORT-ROOT protein, which indicates conserved functions of these proteins. Quantitative RT-PCR analysis shows that the highest level of PrSHR expression is measured in roots. In addition, an induction of the mRNA levels in rooting-competent cuttings was also observed within the first 24 h of the root induction process, however the induction is not dependent on the presence of the exogenous auxin needed for cuttings to root. Changes in the expression profile during adventitious rooting were observed with age and maturation of cuttings. Functional analysis using an Arabidopsis shr mutant is underway.

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Adventitious rooting, SHORT-ROOT, maturation, pine

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Development of SNP marker in putative adaptive relevant genes

Matthias Fladung

Besides air pollution and diseases, climate change is a major threat to tree populations. Short-term responses of tree populations to global environmental changes will depend on already available adaptive diversity. Traditionally, diversity of adaptation is being investigated in provenance tests for a number of forest tree species. These doubtless important studies were mainly focussed on adaptive traits, however, did not provide deep insights into the basic genetic architecture of such complex traits. Despite a number of available publications describing quantitative genetic variation and expectations for adaptation and speciation, very little is known about the molecular basis of adaptation. In view of in the last 10 years developed molecular methods as well as the availability of the annotated genome sequence of Populus trichocarpa, the challenge today is to describe adaptation and/or ability of adaptation at the level of genes or even below.

A key to study the molecular basis of adaptation is the so-called candidate gene approach. Here, genes are marked a priori as possible candidates for the trait of interest (e.g. disease resistance, wood quality, phenological traits, etc.) and the variation is screened in different populations. To reach this aim a Single Nucleotide Polymorphism (SNP) survey will be conducted in candidate genes of different Populus species (e.g. involved in photoperiodic response, lignin biosynthesis, tolerance against biotic and abiotic stresses, etc., available from different data bases. The investigations on the occurrence of SNPs in putatively adaptive genes and related consequences on transcript abundance of these genes will improve our understanding on adaptation and speciation of the tree genus Populus. The results can directly be used to develop molecular makers for adaptation.

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KEYWORDS:
candidate gene; linkage disequilibrium; poplar; adaptation

Faster evaluation of sterility constructs in transgenic trees

Hans Hoenicka, *Matthias Fladung*

A major concern over the use of transgenic trees is the potential for extensive transgene dispersal through pollen and seeds. The incorporation of sterility inducing genes into transgenic lines of trees has been proposed to reduce or even avoid gene flow of transgenes into non-transgenic relatives, which is one of the main ecological concerns with respect to commercial use of transgenic plants. The evaluation of strategies for the induction of sterility in transgenic forest tree species has been hindered by their long vegetative periods. In this study an early flowering 35S::Leafy poplar line was used for the faster evaluation of four different sterility constructs (C-GPDHC::Vst1, C-GPDHC::Barnase, TA29::Barnase, TA29::Vst1). The combination of two transgenic approaches, one to induce early flowering and a second for the induction of sterility, allowed evaluation of this sterility strategy two years after transformation. This is a very short period of time considering the long vegetative period of up to ten to twenty years common in forest tree species. This approach opens new opportunities for the assessment of sterility mechanisms for this plant group.

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KEYWORDS:

sterility in poplar; gene flow

Dynamics of wind-inducible gene expression

Matusikova, C. Olmo, S. Tauber, *S. Fluch*

Plants respond to changes in environment by activating several mechanisms. These include developing and/or strengthening mechanical barriers of cells, changes in levels of intracellular cell components such as calcium, plant hormones and secondary metabolites (e.g. phytoalexins), oxidative burst, triggering expression of different defense genes. The nature, speed and extent of these reactions in the plant remarkably influence the final outcome of adaptation or defense processes. Here we show, that the developmental stage of plant tissue (leaves) is an additional factor that prerequisites the response.

Transcription profiling was performed on poplar leaves of four different developmental stages, while the effect of wind on plants was studied. Out of ~26.000 poplar ESTs existing on microarrays, 210 (about ~ 0,6%) revealed up to 1.7 (log2) fold altered expression in windy conditions. In the two fully expanded, mature leaves analyzed there were 101 and 169 genes differentially expressed respectively. In contrast, 9 genes only were expressed significantly differently ($P<0,01$) in both very young and very old leaves. The list of differentially expressed genes suggests that young and old leaves are better equipped with defense compounds as compared to mature leaves. Our experiments provide first evidence at the transcriptome level that plant tissues of various developmental stages are not equally equipped for or responsive to changes in environment.

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Characterization of cryptochrome blue-light photoreceptors in maritime pine.

M. Ángeles Guevara[1,2], Carmen Collada[2,3], Álvaro Soto[2,4], José Antonio Jarillo[5] and M. Teresa Cervera[1,2]

Cryptochromes are blue/ultraviolet-A light sensing photoreceptors involved in regulating various growth and developmental responses in plants. They were first identified in Arabidopsis and later found in other angiosperms as well as in ferns and algae. However, they had not been described in gymnosperms yet. We report the molecular and functional characterization of pine cryptochrome gene family. To this date, both putative CRY1 and CRY2 orthologue genes have been isolated (named as ppCRY1 and ppCRY2, respectively) and their functional validation have been assayed in transgenic Arabidopsis plants. Overexpression of the predicted ppCRY1 complements the long hypocotyl phenotype of Arabidopsis hy4=cry1 mutation under blue light conditions; indeed, these plants show an extremely short hypocotyl and higher anthocyanin accumulation than hy4 mutant. Transient expression assays of both GFP-CRY1 and GFP-CRY2 fusion proteins in epidermal onion cells by biolistic transformation revealed preferentially nuclear localization of these proteins.

Overexpression analyses of ppCRY2 in transgenic Arabidopsis are underway.

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KEYWORDS:

Cryptochromes; Pinus pinaster; functional characterization; subcellular localization

INTERPOPGER: Interactions between natural and artificial poplar stands and selection pressures associated with their management in French landscape

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In French landscape, poplars are major components of three ecosystems contrasting for the biological resources, local management and public awareness. Populus nigra is an important species of the natural riparian ecosystem when compatible interspecific hybrids are deployed in monoclonal plantations nearby. With its columnar growth habit, Lombardy poplar is undoubtedly the most famous poplar of the landscape. INTERPOPGER project aims at evaluating impact of this land mosaic on genetic diversity of natural black poplar (P. nigra) and on the evolution of genetic diversity of the associated pathogen Melampsora larici-populina (Mlp). Identification of molecular determinants of poplar/Mlp interaction will offer pertinent markers with adaptive value for both organisms. Gene flow from cultivated to natural populations will be quantified at different developmental ages. Impact on gene flow level of landscape components and biological factors such as phenology and fertility will be evaluated. Finally, joint analysis of evolutionary and functional impacts of poplar/Mlp interactions will provide guidelines for sustainable management of natural and cultivated poplar populations.

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KEYWORDS:

Natural resources, land mosaic, gene flow, resistance durability, sustainable management.

C-P12

Genetic Research Of Forest Trees In Lithuania Using Rapd Method

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Random amplified polymorphic DNA (RAPD) method is in general use for plant genetic research. In Lithuanian Forest Research Institute Laboratory of Molecular Genetics and Biotechnology RAPD was used for genetic studies of different forest trees. Twelve plus tree clones of Scots pine (Pinus sylvestris L.), eight Lithuanian populations of Norway spruce (Picea abies (L.) Karst.) and 10 common ash (Fraxinus excelsior L.) populations were studied by RAPD with aim to analyse genetic diversity. Interspecific research between two oaks species (Quercus robur L. and Quercus petraea Liebl.) was accomplished as well. The RAPD technique was also used to check the clones identity of hybrid aspen (P tremuloides x P tremula) plants regenerated in vitro tissue culture and for Norway spruce (Picea abies (L.) Karst.) plus tree clones. Genetic polymorphism of plant regenerants obtained after distance crossing (Populus tremuloides x Fraxinus excelsior) has been confirmed by RAPD as well. The results confirmed that RAPD technique is a useful tool for forest tree genetic variation analysis and for forestry service in managing of clones propagation operations.

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KEY WORDS:

RAPD, genetic diversity, genetic identity, population, hybrid, clone.

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Does a common language exist for root symbioses?

Elena Orsini**, Uwe Nehls#, Ivano Brunner^, Nikolaus Amrhein* and Marcel Bucher

StPT3 is an arbuscular mycorrhiza (AM)-specific phosphate transporter gene from potato (Solanum tuberosum) which is induced at high levels in root cells harboring different mycorrhiza structures, such as arbuscules and thick coiled hyphae (Rausch et al., 2001; Karandashov et al., 2004).

Expression studies using the StPT3 promoter suggested that the mycorrhiza-specific regulation of phosphate transport is conserved within three orders of eudicots: Solanales, Apiales and Fabales (Karandashov et al., 2004).

In the present study, the activation pattern of the StPT3 promoter is investigated in three different symbioses: the endomycorrhizal, the ectomycorrhizal and the nodule symbiosis, respectively, using Populus tremula_tremuloides and Medicago truncatula as model systems.

Endomycorrhized transgenic poplar roots containing the StPT3 promoter fused to the _-glucuronidase (GUS) reporter gene did not show GUS expression, probably because of a lack of intracellular arbuscule and coiled hyphae formation. However, preliminary results showed activation of the StPT3 promoter in poplar roots after vacuum infiltration with a lipophilic extract from mycorrhized Plantago lanceolata roots. In contrast, the ectomycorrhizal fungus Paxillus involutus failed to activate the StPT3 promoter in ectomycorrhized roots of poplar.

Surprisingly, preliminary data with Medicago truncatula plants containing hairy roots carrying the StPT3 promoter fused to the GUS reporter gene showed activation of the promoter in nodules upon inoculation with Sinorhizobium meliloti.

In summary, these results suggest that the pathway leading to StPT3 expression is conserved and inducible not only in herbaceous plants but also in poplar tree. Unlike the evolutionary younger ectomycorrhizal symbiosis, the two endosymbioses, nodulation and arbuscular mycorrhiza, may share common steps in StPT3 promoter regulation.

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Molecular interactions between *Populus & Xanthomonas populi*

A. Pairraud **and JP Métraux**

Poplar (Populus sp.) is one of the major wood ressource in the Northern hemisphere and is a model of choice tree biology. The poplar stem canker, caused by the gram-negativ bacteria Xanthomonas populi pv. populi (Xpp), has an important economical impact on wood production in Europe and is subject to quarantine procedures in North-America.

In the past, several studies has been conducted on genetic and cytological aspects of this disease, but the molecular basis of the pathogenicity remains unclear.
To examine the molecular determinants involved in this interaction, we have developed a foliar system between Xpp and poplar (P. trichocarpa x P. euramerica). This has the advantage to avoid the use of woody tissue to study the Xpp/poplar interaction. Infiltration of poplar leaves with a virulent Xpp strain leads to necrotic lesions while leaves infiltrated with the avirulent strain remain free of macroscopic symptoms. We have characterized our Xpp strains (plasmidic composition, phylogenic position) and we are now developing qPCR approaches to follow the bacterial growth in planta.

The Xanthomonas genus includes hundreds of plant pathogenic species which share a common infection process. It is described as injection of effectors proteins throught the type-three secretion system (TTSS) into the host cell to divert the metabolism of the plant. Some of these effectors, called avirulence factors, can be recognised by the host and trigger defense responses. Based on sequence homology, we have already cloned genes coding for some TTSS components (HrpG, HrpF, HrpX and HpaB) and several putative secreted avirulent factors (AvrBs2, AvrBs3, HpaA, XopJ). We plan to confirm their expression during an infection, and to determine their virulence, cellular localization and biochemical activity.

We would also like to describe the response of poplar leaves at the transcriptional level during compatible or incompatible interactions. We have carried out previous experiments on the expression of several defense-related genes and we are expecting results from microarrays experiments.

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KEY-WORDS :
populus, xanthomonas , stem canker, avr genes

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