ProCoGen final open conference
Promoting Conifer Genomic Resources
30th November – 2nd December 2015
Orléans, France

Conifers are key ecological species dominating many terrestrial landscapes, and they are among the largest terrestrial carbon sinks. Of significant economic importance, conifers are key sources for timber, paper and bio-energy worldwide. At social and scientific levels, there is an increasing awareness of the global change challenges affecting conifers.

In parallel, technological and methodological improvements have been attained and have benefited the conifer taxa, notably on high throughput analytical tools able to describe the variability and plasticity at different levels of integration (from genes up to phenotypes). These new advances can be used not only to improve our understanding of fundamental conifer adaptive biology, but also to address practical problems for the forest industry as well as problems related to the management of conifer forests in the context of global change.

Several international research initiatives have crystalized around these new advances, like next-generation DNA sequencing technologies, with a focus on unraveling fundamental and practical problems of conifer adaptability and domestication. **ProCoGen** is a project funded by the EC 7FP that develops integrative and multidisciplinary genomic research in conifers, using high-throughput platforms for sequencing, genotyping and doing functional analysis. The objective of **ProCoGen** is to unravel genome organization and to identify genes and gene networks controlling important ecological and economic traits, such as those related to environmentally driven tree reaction for growth, drought and cold stress tolerance, and thus provide tree breeders with tools for precise selection. **ProCoGen** as well as other parallel initiatives worldwide have produced already substantial findings deserving broad dissemination among scientist for fostering awareness and further collaboration in conifer research.

With this goal in mind, a **ProCoGen** final open conference will be held in Orleans (France) from November 30th to December 2nd 2015. The aim of this international event will be to serve as a showcase of main results achieved in the project, along with other internationally relevant achievements brought in by key invited speakers and general attendees. External researchers from similar initiatives worldwide, from complementary disciplines ranging from genomics, to molecular and population genetics, tree physiology and developmental biology, biochemistry, molecular and cell biology, bioinformatics and conifer breeders, are invited to present and discuss recent and relevant results on structural, functional, comparative and translational genomics of conifer species. Emphasis will be given to broaden the coverage of key actors, from public research institutes and Universities to privately funded research organizations. External and **ProCoGen** keynote speakers, oral and poster presentations form external attendees and **ProCoGen** members will be included in

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

the program. The number of participants will be limited to 100-120. No registration fees will be demanded. A conference website will be available for registration and abstract submission.

This open conference will be held along with a **ProCoGen** Training Workshop on "*Practicalities of marker and genome-assisted selection*" and with a **ProCoGen** Dissemination Workshop on "*Transfer of genome-related tools to breeding programs*". The TWS and DSW will be held on December 3th 2015 and December 4th 2015, respectively.

ProCoGen final open conference
Promoting Conifer Genomic Resources
30th November – 2nd December 2015
Orléans, France

Centre de Conférences d'Orléans, Orléans, France

Agenda

Monday, November 30th 2015	
11:30 – 12:45	**Registration & Lunch**
Lunch	
13.00 – 13.50	**Opening introduction**
• **I-01** María-Teresa Cervera and Carmen Díaz-Sala, INIA-CIFOR and Universidad de Alcalá (Spain) : Welcoming words from ProCoGen coordinators [ProCoGen] • **I-02** Jean Bousquet, University of Laval (Canada) : Openning conference: current state and future prospects of the conifer genomics [Invited speaker]	

Monday, November 30th 2015	
13.50 – 18.50	**Session 1. Structural genomics of conifer species session1** **Chairman: Pär Ingvarsson (University of Umeå), Yves Van de Peer (Flanders Institute for Biotechnology)**
• **O-01** Nathaniel Street, University of Umeå (Sweden) : Picea abies genome sequencing [Invited speaker] • **O-02** John Mackay, Oxford University (UK) : The genome of white spruce (Picea glauca): draft assemblies, gene family analysis and structural variations [Invited speaker] • **O-03** Jill Wegrzyn, University of Connecticut (USA) : Sequence assembly & annotation in Pinus taeda [Invited speaker] • **O-04** Pär Ingvarsson, University of Umeå (Sweden) : Pinus sylvestris genome sequencing [ProCoGen]	
Coffee break	
• **O-05** Outi Savolainen and Jaakko Tyrmi, University of OULU (Finland) : SNP detection in Scots pine [ProCoGen]	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

• **O-06** Daniel Peterson, Mississippi State University (USA) : Genome sequencing strategies [Invited speaker] • **O-07** Kostya Krutovsky, University of Göttingen (Germany) : Pinus sibirica and Larix sibirica genome sequencing [Invited speaker] • **O-08** Saneyoshi Ueno, Forestry and Forest Products Research Institute (Japan) : Sugi genome resources [Invited speaker] • **O-09** Emily Telfer, Scion (New Zealand) : Challenges assembling large conifer genomes [Selected oral presentation] • Discussion time
Welcome cocktail & poster session

Tuesday, December 1st 2015	
8.30 – 14.50	**Session 2. Functional Genomics of conifer species** **Chairman: Francisco Canovas (University of Malaga), Sara von Arnold (Swedish University of Agricultural Sciences), Luc Harvengt (FCBA)**
• **O-10** Phillip Wilcox, University of Otago (New Zealand) : Genomic and genetic characteristics of the exome capture/transcriptome [Invited speaker] • **O-11** Francisco Cánovas, University of Malaga (Spain) : Pinus pinaster transcriptome dynamics [ProCoGen] • **O-12** Célia Miguel, iTQB (Portugal) : Pinus pinaster miRNAs [ProCoGen] • **O-13** Carl Gunnar Fossdal, Norwegian Forest and Landscape Institute (Norway) : Functional genomics of Picea abies: development and adaptation [ProCoGen]	
Coffee break	
• **O-14** Sara von Arnold, Swedish University of Agricultural Sciences (Sweden) : Transcription regulatory networks associated to development and other key processes [ProCoGen] • **O-15** Jeffrey Dean, Mississippi State University (USA) : Genetic control of adaptive traits in P. taeda [Invited speaker] • **O-16** Jose Celedon, University of British Columbia (Canada) : Functional genomics in white and Sitka spruce and on conifer defense systems [Invited speaker] • **O-17** Luc Harvengt, FCBA Institut Technologique Forêt Cellulose Bois-construction Ameublement (France) : Epigenetic dynamics associated to growth and adaptation [ProCoGen]	
Lunch	
• Selected oral presentations • Discussion time	
15.00 – 19.30	**Session 3. Comparative Genomics of conifer species**

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

	Chairman: Maria-Teresa Cervera (INIA-CIFOR), Lieven Sterck (Flanders Institute for Biotechnology)

- **O-18** Matias Kirst, University of Florida (USA) : *Populus deltoides* genetic and structural variation and its comparison to related species [Invited speaker]
- **O-19** Jérôme Salse, INRA French National Institute for Agricultural Research (France) : Karyotype and gene order evolution from reconstructed extinct ancestors highlight contrasts in genome plasticity of modern rosid crops [Invited speaker]
- **O-20** Jose Antonio Cabezas, INIA-CIFOR (Spain) : ProCoGen comparative mapping based on exome capture [ProCoGen]

Coffee break

- **O-21** Philippe Rigault, Gydle CO (Canada) : Angiosperms analysis: Eucalyptus genome [Invited speaker]
- **O-22** Marina de Miguel, INRA French National Institute for Agricultural Research (France) : Evidence of intense chromosomal shuffling during conifer evolution [ProCoGen]
- **O-23** Pär Ingvarsson on behalf of Amanda de La Torre, Swedish University of Agricultural Sciences (Sweden) : Conifer comparative genome analysis [ProCoGen]
- **O-24** Lieven Sterck, Flanders Institute for Biotechnology (Belgium) : ProCoGen Genome portal: Plaza for gymnosperms [ProCoGen]
- Selected oral presentations
- Discussion time

Wednesday, December 2nd 2015	
8.30 – 15.20	**Session 4. Translational Genomics of Conifer species** **Chairman: Leopoldo Sanchez (INRA), Marco Bink (Wageningen UR)**

- **O-25** Stephen Cavers, NERC Centre for Ecology and Hydrology (UK) : Genomic tools and genetic conservation [Invited speaker]
- **O-26** Jean-Marc Bouvet, CIRAD French agricultural research and international cooperation organization (France) : Multi-trait genomic selection for breeding Eucalyptus hybrids - importance of additive and non-additive effects [Invited speaker]
- **O-27** Marco Bink / Joost van Heerwaarden, Wageningen UR Biometrics (Netherlands) : Optimal uses of genomic information in breeding programs: a simulation study [ProCoGen]
- **O-28** Jérôme Bartholome, INRA French National Institute for Agricultural Research (France) : Integration of molecular tools into the maritime pine breeding program in France [ProCoGen]

Coffee break

- **O-29** Santiago Gonzalez-Martinez, INRA French National Institute for Agricultural Research (France) : Back to nature: candidate genes, population genomics and prediction of maladaptation in natural populations [Invited speaker]

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

• **O-30** John Hickey (UEDIN), University of Edinburgh - Roslin Institute (UK) : Some insights of future developments in GS [Invited speaker] • **O-31** Luc Harvengt, FCBA Institut Technologique Forêt Cellulose Bois-construction Ameublement (France) : Economic evaluation of GS: Maritime pine case study [ProCoGen]	
Lunch	
• **O-32** Giovanni Vendramin, CNR Italian National Research Council (Italy) : Designing core collections from natural range distributions [ProCoGen] • **O-33** John Woolliams, University of Edinburgh - Roslin Institute (UK) : RAD sequencing in Sitka Spruce for Genomic Selection [ProCoGen] • Selected oral presentations • Discussion time	
15.30 – 16.30	**Session 5. Wrap-up presentation by key-note speaker on take-home messages and prospects**
• **O-34** Jean Bousquet, University of Laval (Canada) : Wrap-up [Invited speaker] • Final discussion	
Coffee break & poster session	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

ProCoGen internal meeting

Wednesday, December 2nd 2015	
16.45 – 18.30	**Only for members of ProCoGen consortium**
• Pending activities & future prospects, dissemination and management • General Assembly Meeting (management)	

Social events

Wednesday, December 2nd 2015	
17.00 –	**Social program**
• Old city guided tour (open to all attendants, but parallel to ProCoGen internal meeting) • 20:00 - Social dinner at the restaurant gastronomique *La Vieille Auberge*	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

ProCoGen Training Workshop
Practicalities of marker and genome-assisted selection
3rd December 2015
Orléans, France

Thursday, December 3rd 2015	
8.30 – 12.00	**Practicalities of marker and genome-assisted selection**
Contributors: Marco Bink and Joost van Heerwaarden (Wageningen UR Biometrics, Netherlands), Leopoldo Sanchez and collaborators (INRA, France) [ProCoGen] Illustration of two analytical approaches through the use of freely available tools and real data sets to be provided *in situ* • **TW-01** Marco Bink : Genome-wide association studies	
Coffee break	
• **TW-02** Leopoldo Sanchez : Genomic selection	
Lunch	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

ProCoGen Dissemination Workshop
From our labs to your forests
3rd December 2015
Orléans, France

Thursday, December 3rd 2015	
13.30 – 17.00	**From our labs to your forests**
• **TW-03** Outi Savolainen, University of Oulu (Finland): Using genomics tools to study local adaptation • **TW-04** Concepción Avila, University of Malaga (Spain): Transcriptomic profiles to study development and adaptation in maritime pine	
Coffee break	
• **TW-05** Steve Lee, Forest Research (UK): Genomic selection is here, it's not going away, and it's good for you • **TW-06** Nathalie Isabel, Canadian Forest Service (Québec, Canada) : Translational genomics and its implementation in forestry (*tentative title*)	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

ProCoGen Dissemination Workshop
Transfer of genomic tools to breeding programs
4th December 2015
Orléans, France

Friday, December 4th 2015	
8.30 – 12.00	**Transfer of genomic tools to breeding programs**
• **TW-07** How to start from scratch a genomic characterization (genotyping/sequencing) for a species with little or no sequence resources? Patricia Faivre-Rampant and Aurélie Bérard [INRA] • **TW-08** Genetic mapping and QTL meta-analysis, a review through a demo of BioMercator, Yannick de Oliveira [INRA] • **TW-09** How to build up a core collection from natural ranges, with the aim of compiling a species reference collection, a discovery panel, or a base for breeding? Giovanni Vendramin [ProCoGen]	
Coffee break	
• **TW-10** Can genomics simplify current breeding without the cost of a genome-wide approach? Laurent Bouffier [INRA] • **TW-11** Why and how to handle genetic diversity in a genomic selection program? Leopoldo Sanchez [INRA]	
Lunch	

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

Picea abies genome sequencing

Nathaniel Street [Invited speaker] The Norway spruce genome project

University of Umeå (Sweden)

We sequenced the Norway spruce (*Picea abies*) genome using a hierarchical approach that combined whole genome shotgun and fosmid pool sequencing data. While the resultant assembly was highly fragmented, the gene space was reasonably well represented allowing gene annotation, which identified 28,354 high quality gene models in addition to 42,434 lower quality loci. These low quality loci represent both genuine gene models that remain fragmented in the assembly in addition to a large proportion of pseudogene fragments. Many genes contained long introns as a result of LTR element insertions, many of which are phylogenetically conserved among coniferous species, suggesting an ancient and conserved point of origin. This pattern was present at the genome level, with phylogenetic analysis of LTR element families across a range of coniferous species identifying an ancient and shared origin. Repeat elements were often present as singletons, suggesting low rates of activity yet there was an abundance of complete elements, suggesting a lack of removal due to unequal recombination. In parallel, significant differences were highlighted between the 24nt sRNA population of Norway spruce compared to angiosperm and basal plant species. This class of sRNA are involved in silencing of repeat elements via RNA-dependent DNA methylation.

Since publication of the Norway spruce genome, our data has been used by many other research groups to further advance insight into genome evolution, sRNA analyses and to aid phylogenetic insight. I will overview some of these as well as overviewing our own ongoing research and plans for future assembly improvement and resource development.

The genome of white spruce (*Picea glauca*): draft assemblies, gene family analysis and structural variations

R. L. Warren[1], C. I. Keeling[2], J. Prunier[3], A. Sahli[3], S. Caron[3], M. M. Saint Yuen[2], A. Raymond[1], G. A. Taylor[1], B. P. Vandervalk[1], D. Paulino[1], G. Robertson[1], C. Yang[1], C. Ritland[4], A. Yanchuk[5], J. Bousquet[3], S. J. Jones[1,6,7], I. Birol[1,6,7,], J. Bohlmann[2,3,8], J. J. MacKay[3,9]

[1]Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada;
[2]Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada;
[3]Department of Wood and Forest Sciences, Université Laval, Québec, QC, Canada;
[4]Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada;
[5]British Columbia Ministry of Forests, Lands, and Natural Resource Operations, Victoria, BC, Canada;
[6]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada
[7]School of Computing Science, Simon Fraser University, Burnaby, BC, Canada;
[8]Department of Botany, University of British Columbia, Vancouver, BC, Canada
[9]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, UK

Progress will be reviewed for white spruce (*Picea glauca*) genome sequencing and assembling, and insights will be presented from targeted investigations into gene family structure and structural variation.

Draft genome assemblies were obtained for two white spruce genotypes, PG29 and WS77111 that originate from distant geographic regions of western (PG29) and eastern (WS77111) North America. Assemblies of the PG29 and WS77111 genomes confirm the reconstructed white spruce genome size in the 20 Gbp range, and show broad synteny.

Using the PG29 assembly and additional white spruce genomics and transcriptomics resources, we performed *ab initio* MAKER-P annotation and meticulous expert annotation of very large gene families of conifer defense metabolism, terpene synthases and cytochrome P450s. We also comprehensively annotated the white spruce mevalonate, methylerythritol phosphate and phenylpropanoid pathways. These analyses highlighted the large extent of gene and pseudogene duplications, in particular for genes of secondary (i.e. specialized) metabolism, and the potential for gain and loss of function for defense and adaptation.

We report on two lines of investigation to shed light into genomic structural variations at the intraspecific level. Our analyses used genotyping data obtained from large-scale analyses of families and structure populations and have tailored developed array CGH analysis methods applied to nuclear families. Large numbers of heritable copy number variations as well as de novo mutations were found. The high frequencies and the functional annotations of genes affected indicated that structural variations may have an significant impact that merits further analyses of their role in adaptation and molecular marker development.

We discuss future directions for conifer genome analysis as relevant for developing a more integrated understanding of genome function and variations.

The genome sequence of sugar pine and Pinus genome evolution

J.L. Wegrzyn[1][Invited speaker], K.A. Stevens[2], R. Paul[1], D. Gonzalez[1], U. Uzay Sezen[1], A. Zimin[4], D. Puiu[8], M. Crepeau[2], C. Cardeno[2], M. Koriabine[5], A.E. Holtz-Morris[5], P.J. Martínez-García[3], E. Grau[1], H.A. Vasquez-Gross[3], K. Jermstad[7], G. Marçais[4], M. Roberts[4], C. Holt[10], M. Yandell[10], P.E. McGuire[2], D. Main[12], C.A. Loopstra[11], P. deJong[5], A. Eckert[6], J.A. Yorke[4], S.L. Salzberg[8], K. Mockaitis[13], C.H. Langley[2], D.B. Neale[3]

[1]Department of Ecology and Evolutionary Biology, Univ. of Connecticut, Storrs, CT
[2]Department of Plant Sciences, University of California at Davis, Davis, CA
[3]Department of Evolution and Ecology, University of California at Davis, Davis, CA
[4]Institute for Physical Sciences and Technology (IPST), University of Maryland, College Park, MD
[5]Children's Hospital Oakland Research Institute, Oakland, CA
[6]Department of Biology, Virginia Commonwealth University, Richmond, VA
[7]United States Forest Service, Placerville, CA
[8]Center for Computational Biology, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD
[10]Department of Human Genetics, University of Utah, Salt Lake City, UT
[9]Departments of Mathematics and Physics, University of Maryland, College Park, MD
[11]Department of Ecosystem Science and Management, Texas A&M University, College Station, TX
[12]Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA
[13]Department of Biology, Indiana University, Bloomington, IN

The diploid genome of Sugar pine (Pinus lambertiana) has an estimated genome size of 31 Gb, with repetitive content estimates exceeding 80% of the genome. With nearly 50 percent more nuclear DNA than the current record holder, Pinus taeda (Neale et al. 2014), it is notable as the largest genome sequenced and assembled to date. The assembly, annotation, and related gene space characterization of sugar pine, the sole member of the subgenera Strobus, represent extensive efforts towards describing this resource.

The crux of our approach has been to leverage an aspect of conifer biology to reduce the complexity of the assembly problem. We have found that the limited amount of haploid DNA obtainable from of a single megagametophyte can be used to create an ensemble of libraries of sufficient complexity to form the basis of a high quality whole genome shotgun assembly. The resulting haploid sequence data is well suited to additional computational methods for reducing the complexity of the assembly problem. The challenges of the larger genome necessitated additional developments to the assembly procedure.

Comparative analysis of sugar pine and loblolly pine genomic resources, reveals new insights on the conservation, age, and diversity of the highly abundant transposable elements, a primary factor determining the genome size. Analysis of the gene space between pine and spruce species has identified conifer-specific genes, expanded families, insight on pseudogenes, and the potential relationships between transposable elements and gene expression. Genomic observations are enabled through an independent and thorough characterization of the sugar pine transcriptome.

The principal pathogen of P. lambertiana is white pine blister rust (Cronartium ribicola). Of great interest is identifying a molecular marker and candidate gene for the major dominant gene for simple resistance hypersensitive response (Cr1). Combined genomic resources assisted in the validation and development of new markers, extending the existing genetic map resources for Cr1. Genomic and transcriptome resources also reveal new insights regarding additional genes coding for antifungal agents critical to the defense pathways in sugar pine.

Pinus sylvestris genome sequencing

Pär Ingvarsson

University of Umeå (Sweden)

SNP detection in Scots pine

Outi Savolainen[1], Jaakko Tyrmi[1], Soile Alatalo[1], Tanja Pyhäjärvi[1], Jaana Vuosku[1], Lieven Sterck[2], Zhen Li[2], Juan Acosta[3], Matias Kirst[3]

[1] Dept Genetics and Physiology, University of Oulu
[2] Department of Plant Systems Biology, VIB
[3] School of Forest Resources and Conservation, University of Florida

Exome capture was used to discover SNPs in Scots pine. The first experiment was using probes designed based on *P. taeda* from Neves et al. 2013. The target specificity in the capture was fairly low, and capture of paralogues was common. SNP calling and filtering were applied using a custom made pipeline. This experiment yielded several thousand SNPs in a set of European main distribution wide samples. The Scots pine transcriptome was used as the basis of the next probe design. An initial set of 160 000 probes was reduced to 60 000, based on mapping against the *P. taeda* genome and empirical results. Specificity of capture for this more limited set still was not very high, but even sequencing coverage on target areas was achieved. Stringent filtering of haploid samples yielded high quality SNPs that allow population genetic analyses.

Genome Sequencing and Assembly Strategies

Dr. Daniel G. Peterson
Director

Institute for Genomics, Biocomputing & Biotechnology (IGBB), Mississippi State University, Mississippi State, MS 39762 USA

Until recently, whole genome sequencing was financially impracticable to all but those large scientific communities studying model and/or economically-important organisms. Such sequencing was expensive as it was rooted in physical mapping of large-insert DNA clones, elucidation and sequencing of minimum tiling paths, and a utilization of various gap filling and scaffold-building techniques. However, the advent of 2nd generation DNA sequencing technologies (e.g., 454/Roche, Illumina, and IonTorrent) – and consequent decreases in sequencing costs – theoretically made whole genome sequencing an affordable option for even small groups of scientists including those lacking physical and/or molecular linkage maps for their species of interest. While 2nd generation sequencing has certainly accelerated the study of genetic diversity and gene expression, its potential as a whole genome sequencing tool has been rather disappointing. For a few thousand dollars, almost anyone can initiate a genome sequencing project (i.e., generate billions of nucleotides of sequence data), but the process of assembling pseudomolecules from 2nd generation sequence data is far from democratized. Moreover, genomes assembled from only 2nd generation sequence data are less likely to reflect biological reality than those genomes produced using the more expensive, physical map-based strategies of yore. I will discuss past and current genome sequencing strategies and recent advances that look to make whole genome sequencing and assembly more accurate. In addition, I will highlight areas that still merit further attention. Sugi genome resources

Pinus sibirica and *Larix sibirica* whole genome *de novo* sequencing

Krutovsky K.V.[1,2,3,4*], Oreshkova N.V.3[,5], Putintseva Yu.A.[3], Kuzmin D.A.[3], Pavlov I.N.[3,5], Sharov V.V.[3], Biryukov V.V.[3], Makolov S.V.[3], Deych K.O.[3,6], Bondar E.I.[3], Ushakova O.A.[3], Ibe A.A.[3,6], Shilkina E.A.[6], Sadovsky M.G.[7], Vaganov E.A.[3]

1University of Göttingen, Göttingen, Germany
2N.I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia
3Siberian Federal University, Krasnoyarsk, Russia
4Texas A&M University, College Station, Texas, USA
5V.N. Sukachev Institute of Forest, Siberian Branch of Russian Academy of Sciences, Krasnoyarsk, Russia
6Forest Protection Centre of Krasnoyarsk Territory, Krasnoyarsk, Russia
7Institute of Computational Modeling, Siberian Branch of Russian Academy of Sciences, Krasnoyarsk, Russia
*e-mail: konstantin.krutovsky@forst.uni-goettingen.de

The Siberian larch (Larix sibirica Ledeb.) and Siberian pine (Pinus sibirica Du Tour.) nuclear and organelle genomes are being de novo sequenced in the Laboratory of Forest Genomics at the Genome Research and Education Center of the Siberian Federal University using Illumina HiSeq 2000 and MiSeq, and their first draft genome assemblies were generated (http://genome.sfu-kras.ru/en/main). Estimated genome size was 12.03 Gbp for Siberian larch and 28.90 Gbp for Siberian pine. DNAs isolated from needles, single megagametophytes and a haploid tissue culture of a reference larch tree and from needles and single megagametophytes of a reference pine tree were used to generate multiple PE libraries with 250, 400 and 500 bp long inserts and MPE libraries representing 3 and 5 Kbp long fragments. We tested CLC Assembly Cell, ABySS and MaSuRCA assemblers that were used in the similar Picea abies, Picea glauca and Pinus taeda conifer genome sequencing projects, respectively. The assembling was done using the IBM x3950 x6 server with 96 cores and 3 TB RAM. Based on our results ABySS was the most stable, but the best assemblies were generated by CLC Assembly Cell. The best Siberian larch genome assembly was ~5.5 Gbp long (that is 46% of the expected complete genome length) with N50 for contigs equaled 1947 bp. Almost all Siberian pine short reads were successfully mapped to the draft genome assembly v1.0 of closely related sugar pine (Pinus lambertiana Dougl.) generated in the PineRefSeq project (http://pinegenome.org/pinerefseq) covering more than 80% of the assembly (~21.26 Gbp). Thus, the reference-based together with de novo assembly approaches resulted in a draft genome assembly of Siberian pine with a total length of ~22.9 Gbp (79% of the expected complete genome length) with N50 for contigs equaled 2352 bp. About 80% of Siberian larch and pine nuclear genomes consisted of highly repetitive DNA. We searched for microsatellite loci in the assemblies and designed PCR primers to identify and develop many highly polymorphic and informative SSR-markers for population genetic studies. For the first time the chloroplast genome of Siberian larch has been assembled and annotated. For Siberian pine we improved the chloroplast genome assembly published in Genbank (FJ899558.1) by closing gaps with the total gap length of 16085 bp. The draft assemblies of mitochondrial genomes for these and a few other conifer species have been also generated. The larch transcriptome assembly consisted of 43717 unigenes with a total length of ~26 Mbp. The longest unigene was 8512 bp; N50 = 1330 bp, and the number of unigenes longer than 1 Kbp was 6919. The obtained transcriptome assembly represented ~70% of the estimated total transcriptome in Siberian larch and was similar to other published conifer transcriptomes. This study was supported by Research Grant No. 14.Y26.31.0004 from the Government of the Russian Federation.

Sugi genome resources

Saneyoshi Ueno[1], Yoshinari Moriguchi[2], Kentaro Uchiyama[1], Tokuko Ujino-Ihara[1], Asako Matsumoto[1], Norihiro Futamura[1], Yoshihiko Tsumura[3]

[1]FFPRI, [2]Niigata University, [3]University of Tsukuba

Sugi (*Cryptomeria japonica*) is a coniferous tree native to Japan and the most important forestry species, the plantation of which covers 4.5 million ha and amounts to 44 % of artificial forests of the country. Unfortunately pollen of sugi has developed allergy, causing more than one third of Japanese sugi-pollinosis. Economic losses caused by the disease are estimated to be hundreds of billions of yen per year. Therefore the present genetic and genomic research of *C. japonica* in our institute is focused on understanding the genetic architecture of 1) pollen production to breed male sterile trees and 2) environmental adaptation in relation to growth, timber quality and pollen production. Integration of these two topics will help develop new varieties of excellent sugi with no pollen dispersal.

In this talk, we introduce our recent efforts to establish genetic and genomic resources of sugi, including cDNA and BAC libraries, genetic markers, mapping populations and ForestGEN database. These resources have been used for mapping male sterility genes and QTLs concerning growth characteristics and pollen production.

Challenges assembling large conifer genomes

Emily Telfer, Lucy Macdonald, Natalie Graham, Tatiana Lomasko and Heidi Dungey

Scion, 49 Sala St, Rotorua, New Zealand

We review the last three years work sequencing and assembling the large 25 Gb genome of Pinus radiata and present options for overcoming the challenges presented by complex genetic architecture with large multi-gene families and an extensive repeatome. This project sits alongside an industry aligned programme to generate and develop SNP genotyping resources for both genomic selection (prediction of future performance) and pedigree reconstruction (elucidation of parentage from within mixed seedlots). After generation of raw data at approximately 30 x depth, we initiated a reference-based assembly using the Pinus taeda v1.01 genome release as a reference. However, the sheer size of both the reference (21 Gb) and target (25 Gb) has proven problematic. In addition, the limited supply of haploid tissue (megagametophyte), an essential experimental component of assembling a large heterogeneous genome, has hampered our ability to utilise third generation sequencing platforms such as PacBio. We present a strategy for future work and a roadmap to a working resource for the radiata pine and conifer community.Pinus pinaster transcriptome dynamics

Using transcriptomes for developing DNA marker resources in *Pinus radiata* D.Don and endemic New Zealand *Podocarpus*

Phillip Wilcox[1] [Invited speaker], Emily Telfer[2], Natalie Graham[2], Scion, Lucy Macdonald[2], Heidi Dungey[2], Yongjun Li[2], David Chagné[3], David Bergin[4], Christina Marshall[5], John McCallum[3], Peter Lockhart[5]

[1] Department of Statistics and Mathematics, University of Otago, New Zealand
[2] Scion, Rotorua, New Zealand
[3] Plant and Food Research Ltd, Christchurch, New Zealand
[4] Environmental Restoration Ltd., Rotorua, New Zealand
[5] Massey University, Palmerston North, New Zealand

The relatively large size of conifer genomes and the costs of sequencing and assembly have precluded the rapid development of reference genome sequences. In contrast, transcriptome sequences are relatively cheap to obtain and assemble, and can (a) directly provide polymorphism data in genic regions, and/or (b) provide a reference for gDNA re-sequencing that specifically targets functional genes. We have used these approaches to develop genome-wide DNA markers in *Pinus radiata* D.Don, a commercially important forest tree species in New Zealand (NZ), and also in NZ-endemic species of *Podocarpus*. For *P. radiata* we generated cDNA sequence derived from up to 10 different tissue types that had been collected from 8 genotypes. A *de novo* assembly using Trinity RNASeq yielded contigs corresponding to 194 299 gene fragments containing approximately 329 000 SNPs. Alignment to the v1.01 *P. taeda* L. assembly using TopHat followed by BLASTn-based gene prediction indicated approximately 450 000 exons. Using this resource, an exome capture panel has been developed and evaluated in collaboration with Rapid Genomics LLC. Initially, an 80K probe panel was designed. Subsequent testing in several populations, including two full sib families, reduced the panel to approximately 44K probes. After filtering of individual data points, 961 550 SNPs were identified. Preliminary results from linkage mapping analyses using a previously constructed linkage map showed virtually all segregating SNPs mapped to the expected twelve linkage groups. Further research using this panel is planned, including prediction of genomic breeding values in breeding populations. We have also developed high resolution melting (HRM) assays to differentiate among endemic New Zealand *Podocarpus* species. Using an ultra-low coverage *de novo* assembly of genomic sequence from *Podocarpus totara* G.Benn ex. D.Don, contigs containing protein-encoding genes were identified via BLASTn analyses using *Pinus taeda*, *Picea glauca*, *Picea sitchensis*, and *Cryptomeria japonica*-derived EST data bases. We then designed and evaluated 120 HRM primer pairs, yielding ten pairs that differentiated all four endemic New Zealand *Podocarpus* species as well as one Australian endemic. Collectively these studies indicate that transcriptomic data yield useful information that expedites DNA marker panel development.

Pinus pinaster transcriptome dynamics

Francisco M. Cánovas, Concepción Avila, Rafael Cañas, Fernando de la Torre, Belén Pascual, Vanessa Castro, Jorge El-Azaz

Grupo de Biología Molecular y Biotecnología de Plantas
Departamento de Biología Molecular y Bioquímica, Universidad de Málaga-SPAIN

A wide range of genomic-based approaches have been used in the ProCoGen project to study the functional regulation of growth and adaptive responses in European pines. Maritime pine (*Pinus pinaster* L. Aiton) is a conifer species with great economic and environmental value that is widely distributed in the south-western area of the Mediterranean region, dominating forests in France, Portugal, and Spain. It has high phenotypic plasticity, accompanied by high tolerance to abiotic stresses such as drought, and widely distributed in varying environments. In the frame of the functional genomics studies included in the workpackage 2 of the ProCoGen project, transcriptome dynamics has been explored in *Pinus pinaster*. A map of transcriptional activity of the maritime pine tissues has been established by massive paralell sequencing analysis of tissue-specific transcriptomes. The analysis of the data revealed significant differences in the total number of expressed genes in distinct cell and tissues. This map of transcriptional activity complement previous studies on the maritime pine transcriptome (Canales et al., 2013) and represents a valuable new resource for the functional characterization of gene families and transcription factors associated to growth and adaptive responses. Seasonal and developmental changes in transcriptomic and metabolomics profiles have also been examined in needles of adult maritime pine trees growing under natural conditions. Interesting gene co-expressing networks have been identified and the functional analysis of relevant candidate genes in underway. An overview this research programme will be presented and discussed.

Canales J, Bautista R, Label P et al. (2014) De novo assembly of maritime pine transcriptome: implications for forest breeding and biotechnology. *Plant Biotechnol Journal*, 12, 286-299.

Physiological and molecular responses to drought in maritime pine (*Pinus pinaster* Ait).

Nuria de María et al.

Departamento de Ecología y Genética Forestal. INIA-CIFOR. Ctra. de La Coruña Unidad Mixta de Genómica y Ecofisiología Forestal, INIA/UPM, Madrid, Spain

Climate change predictions point at increasing dryness over the Mediterranean region compromising growth and survival of forest trees. Pinus pinaster, which is found in a relatively small geographical range along a rainfall cline, is characterized by a significant genetic and adaptive diversity. Drought response is a complex trait that needs to be considered at a global systems biology level to study the multiple interactive components.
Different approaches have been used to analyze Pinus pinaster response to drought, studying a progeny from an ad-hoc designed full-sib cross (Gal1056 x Oria6). Progeny individuals showed high variability in their response to drought (i.e osmotic adjustment capacity), showing moderate heritability values for stomatal conductance and intrinsic water use efficiency. Both cDNA and miRNA libraries were constructed representing different tissues and growing conditions from clonally propagated individuals. Differential expression analysis has been used to identify potential candidate genes further subjected to qRT-PCR. Additionally, a 1,536 SNP array has been developed based on SNPs associated to the mapping population and used to construct, together with additional SNPs, dense genetic maps (de Miguel et al. 2012 BMC Genomics; de Miguel et al. 2014 BMC Genomics). These maps are being saturated using an exome capture system designed by ProCoGen for conifer comparative mapping. Genetic maps have also been used for the dissection of leaf gas exchange, chlorophyll fluorescence parameters and water use efficiency in response to drought, detecting QTLs that explained 10-20% of the observed phenotypic variability for each trait. Additionally, DNA cytosine methylation as well as untargeted analysis of metabolic profiles allowed the discrimination of genotypes with contrasting drought response. Different metabolites, such as glutamate family amino acids, polyols and lipids, correlated with some of the ecophysiological traits responding to drought.

Pinus pinaster miRNAs

Andreia Rodrigues[1,2], Inês Chaves[1,2], Susana Lopes[1], Bruno Costa[1], Andreas Bohn[1,2], Célia Miguel[1,2*]

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. República, 2780-157 Oeiras, Portugal
[*] cmiguel@itqb.unl.pt"

Small non-coding RNAs (sRNA) are major regulators of gene expression during plant growth and development, and response to abiotic and biotic stresses. Little is known about the sRNA transcriptome of gymnosperms and its putative role in the distinct characteristics exhibited by these species when compared to the angiosperms. The recent release of the first gymnosperm genome sequences (1,2) provides an important resource to further investigate sRNAs in these species.
We have profiled the sRNAs of Pinus pinaster in a wide range of tissues, developmental stages and under drought stress. The use of an in-house sRNA pipeline (https://github.com/forestbiotech-lab/sRNA-workflow) for the global analysis of the sequencing data obtained so far allowed identifying over 30 families of conserved microRNAs (miRNA) and prediction of novel miRNAs. Several approaches have been followed to validate a set of the predicted miRNAs and their target genes. We then focused on the developing embryo miRNA transcriptome as it has been suggested that differential gene regulation rather than the presence of the gymnosperm specific genes may be responsible for the distinct features exhibited during embryo development (3). A differential representation of the 24nt (typically comprising siRNAs) and the 20-22nt sRNAs (typically comprising the miRNAs) was found in the five developmental stages analysed, showing opposite abundance trends along embryo development.

Acknowledgements
FCT for grant SFRH/BD/79779/2011 (AR) and EU for project PROCOGEN nº 289841.
1. Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin Y-C, Scofield DG, Vezzi F et al. (2013) Nature 497: 579–584.
2. Neale DB, Wegrzyn JL, Stevens KA, Zimin AV, Puiu D, Crepeau MW, Cardeno C et al. (2014) Genome Biol 15(3):59-59.
3. de Vega-Bartol JJ, Simões M, Lorenz WW, Rodrigues AS, Alba R, Dean JFD, Miguel CM (2013) BMC Plant Biol 13:123.

Functional genomics of Picea abies: development and adaptation

Carl Gunnar Fossdal

Norwegian Forest and Landscape Institute (Norway)

Transcription regulatory networks associated to development and other key processes

Sara von Arnold, Irene Merino, Malin Abrahamsson.

Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO-BOX 7080, SE-75007 Uppsala, Sweden.

In conifers it is common that multiple, equal-sized embryos develop from a single zygot by a process termed cleavage polyembryony. These embryos start to compete and one embryo becomes dominant, while the remaining embryos, called subordinate embryos, are degraded by programmed cell death. The aim of this study has been to get information about the regulation of the cleavage process and the development of the dominant embryo. Our approach has been to: (i) identify transcripts differentialy expressed in early stages during zygotic embryogenesis by transcriptome analyses, (ii) to confirm the expression of putative candidate genes by qRT-PCR analyses and (iii) to elucidate the function of candidate genes by over- and/or under-expressing the genes during development of somatic embryos. The developmental stages included in the study have been: single zygote derived early embryos, multiple embryos at equal size after cleavage, dominant and subordinate embryos. In addition, the megagametophyte at each developmental stage were analysed separately.
In total, around 80.000 transcripts (RPKM> 0) were detected. Out of which 26% were only detected in embryos and 9% only in megagametophytes. About 35% of the transcripts showed homology with genes in the Arabidopsis thaliana TAIR database. GO enrichment analyses pointed out the importance of cell cycle, cell differentiation, cell growth and developmental processes in the embryos while accumulation of storage products and catalytic activities were important in the megagametophytes.The expression of differentially expressed transcripts during embryo development has been confirmed by qRT-PCR in three to four biological replicates. Based on these results we have started to identify critical processes during early embryo development in Scots pine.

This work was supported by the European Community´s Seventh Framework Programme under agreement No. 289841 (Project ProCoGen).

Genetic control of adaptive traits in *P. taeda*

Jeffrey Dean [Invited speaker]

Mississippi State University (USA)

Functional genomics in white and Sitka spruce and on conifer defense systems

Jose Celedon [Invited speaker]

University of British Columbia (Canada)

Epigenetic dynamics associated to growth and adaptation

Luc Harvengt[1], Carmen Diaz-Sala[2], Maite Cervera[3], Grégoire LeProvost[4], and Carl Gunnar Fossdal[5]

[1] FCBA Institut Technologique Forêt Cellulose Bois-construction Ameublement (France)
[2] University Alcala de Henares, Spain
[3] INIA-CIFor, Madrid, Spain
[4] INRA, France
[5] NIBIO (previously known as NFLI), Norway

Epigenetic marks have been investigated in Maritime pine full sib famillies subjected to contrasted levels of artificial drought stress. The same plant material was used in other tasks of the project, mainly transcriptomic studies. P1-UAH & P2-INIA investigated the occurrence of differential methylation in cuttings from a cross between parents from natural population from Spanish regions of contrasted climate. Random methylationsenisitive amplification uncovered some hundred anonymous loci experiencing methylation changes during or after the completion of artificial drought stress. P4-INRA studied methylation change at specific loci uncovered through a comprehensive transcriptomic and metabolomic approach in an intensively characterized Landes x Morocco mapping family (results will be lately available). P5-FCBA generated epigenetically –induced maritime pine somatic embryos of a model genotype already extensively characterized at metabolomics and transcriptomic level for further studies. P14-NFLI/NIBIO characterized sRNAs, miRNA and their target mRNA in Norway spruce plant material subjected to contrasting epigenetic-inducing treatment during somatic embryogenesis. Relationships with further plant development and chromatin modification (appreciated through ChIP-Seq)) will be presented.

Populus deltoides genetic and structural variation and its comparison to related species

Annette M. Fahrenkrog[1,2], Christopher Dervinis[1], William B. Barbazuk[3,4], Matias Kirst[1,2,4]

[1]School of Forest Resources and Conservation, University of Florida, P.O. Box 110410, Gainesville, FL 32611, USA
[2]Plant Molecular and Cellular Biology Graduate Program, University of Florida, P.O. Box 110690, Gainesville, FL 32610, USA
[3]Biology Department, University of Florida, P.O. Box 118525, Gainesville, FL 32611, USA
[4]University of Florida Genetics Institute, University of Florida, P.O. Box 103610, Gainesville, FL 32611, USA

The genus *Populus* includes a large number of species and hybrids that are explored commercially, most recently for bioenergy production. Of the different species, *P. deltoides* represents one of the most intensively cultivated. In order to begin unraveling the genome of the species, we recently re-sequenced a large fraction of the coding sequence of the genome of 579 unrelated individuals by target-capture. Oligonucleotides that tile regulatory and coding sequences of all poplar genes previously identified as expressed in the main vegetative tissues were designed. These were shown to be effective for capture and genotyping of genomic regions that include exons and parts of the 5' and 3' untranslated regions (UTRs) of 18,153 genes. Analysis of the entire population identified a set of 535,532 single nucleotide polymorphisms (SNPs) in the nuclear genome, as well as a large number of indels. Additionally, off-target reads allowed the detection of SNPs in chloroplasts and mitochondria. Depth of sequencing was also analyzed to identify large structural variants – particularly gene gain and loss. The genotypic data is being compared to recent reports in related taxa, for identification of homologous loci that contribute to critical developmental traits such as sex-determination.

Paleogenomics : Unraveling modern species adaptation from extinct ancestors.

Jerome Salse

UMR INRA-UBP 1095 GDEC, Laboratoire Paleogénomique & Evolution (PaleoEVO), 5 Chemin de Beaulieu, 63100 Clermont-Ferrand, FRANCE
e-mail : jsalse@clermont.inra.fr

During the last decade, technological improvements in sequencing technologies led to the development of large sets of genomic resources permitting the emergence of high-resolution comparative genomic studies in both plant and animal lineages. Paleogenomics research, aiming at reconstructing ancestral genomes of modern living species, allowed us to propose a model in which the plant and animal genomes have evolved from a common ancestors with respectively a basic number of 7 to 13 chromosomes through whole genome duplications (i.e. paleopolyploidization) and translocations followed by lineage-specific segmental duplications, chromosome fusions and translocations. These data demonstrates how extant animal and plant genomes are the result of inherently different rates and modes of genome evolution resulting in relatively stable animal and much more dynamic and plastic plant genomes. The established ancestral genomes, in term of chromosome structure and gene content, offer the opportunity to perform high resolution translational research from models and species of agronomic/medical interest.

ProCoGen comparative mapping based on exome capture

José Antonio Cabezas[1], Nuria de Maria[1], Ahinoa Calleja-Rodríguez[2], Amaryllis Vidali[2], Carolina Bernhardsson[2], Joan Cotrell[3], Stuart A'Hara[3], Sylvie Blais[4], Natalie Isabel[4], Christophe Boury[5], Marina de Miguel[1,5], Zhen Li[6], Jaakko Tyrmi[8], Lieven Sterck[6], Christophe Plomion[5], Jean Bousquet[4], Steve Lee[3], Outi Savolainen[7], María Rosario García Gil[2], Pär Ingvarsson[2], María Teresa Cervera[1]

[1] Centro de Investigación forestal (INIA-CIFOR), Madrid, Spain; [2] Umeå Plant Science Centre, Umeå, Sweden; [3] Forest Research, Northern Research Station, Roslin, United Kingdom; [4] Universite Laval, Quebec, Canada; [5] Institut National de la Recherche Agronomique, UMR 1202 BIOGECO, Cestas, France; [6] Ghent University, Ghent, Belgium; [7] University of Oulu, 90014 Oulu, Finland

One of the tasks developed in the European project ProCoGen is the comparative genetic mapping among different model conifer species. The basic scheme was: 1) to develop a single exome capture system integrating information from different *Pinus* and *Picea* species; 2) to use this tool to genotype 17 mapping populations from *P. pinaster*, *P. sylvestris*, *Picea abies*, *P. sitchesis*, and *P. glauca*; 3) to use this information to construct the genetic maps for each progeny; 4) and to subsequently combine the progeny maps to generate a composite linkage map for each species that will be used for comparative mapping.

The probes designed for genotyping included in the final exome capture system were selected from a pilot 57K exome capture that targeted putative orthologue sequences from two sets of data. The first set consisted on 27,558 probes obtained from sequence comparisons among the genomic information available for 6 species (3 spruces and 3 pines). The second set consisted of 29,450 probes selected from a previous *P. pinaster* and *P. sylvestris* analysis using a *P. taeda* exome capture system (Neves et al. 2013). In order to select the most informative probes across the whole range of species, the 57K exome capture was used to genotype the progenitors of all the mapping progenies in the study and three megagametophytes of each of 5 species. The analysis of the progenitors provided information about informative probes, whilst the analysis of the haploid DNAs allowed probes targeting putative paralogous sequences to be discarded (reported as misleading heterozygous SNPs). The final 10K exome capture design, which includes 9,622 probes, was used to genotype the 17 mapping progenies with the results used to construct genetic maps for each progeny and ultimately the corresponding composite maps for each species.

Angiosperms analysis: Eucalyptus genome

Philippe Rigault [Invited speaker]

Gydle CO (Canada)

Evidence of intense chromosomal shuffling during conifer evolution

Marina de Miguel[1,2,$], Jérôme Bartholomé[1,2,$], François Ehrenmann[1,2], Florent Murat[3], Yoshinari Moriguchi[4], Kentaro Uchiyama[5], Saneyoshi Ueno[5], Yoshihiko Tsumura[6], Hélène Lagraulet[1,2], Nuria de Maria[7,8], José-Antonio Cabezas[7,8], Maria-Teresa Cervera[7,8], Jean Marc Gion[1,2,9], Jérôme Salse[3], Christophe Plomion[1,2]

[1] INRA, UMR 1202 BIOGECO, 69 Route d'Arcachon,F-33610 Cestas, France
[2] Université de Bordeaux, UMR 1202 BIOGECO, F-33170 Talence, France
[3] INRA/UBP UMR 1095 GDEC 'Génétique, Diversité et Ecophysiologie des Céréales', 5 Chemin de Beaulieu, 63100 Clermont Ferrand, France
[4] Niigata University, Graduate School of Science and Technology, 8050, Igarashi 2-Nocho, Nishi-ku, Niigata 950-2181, Japan
[5] Forestry and Forest Products Research Institute, Department of Forest Genetics, Tsukuba, Ibaraki 305-8687, Japan
[6] University of Tsukuba, Faculty of Life & Environmental Sciences, 1-1-1, Tennodai, Tsukuba, Ibaraki 305-8572, Japan
[7] INIA-CIFOR, departamento de Ecologia y Genetica Forestal, 28040, Madrid, Spain
[8] INIA-UPM, Unidad mixta de Genomica y Ecofisiologia Forestal, Madrid, Spain
[9] CIRAD, UMR AGAP, F-33612 Cestas, France
[$]contributed equally

While recent advances have been gained on genome evolution in angiosperm lineages, virtually nothing is known about karyotype evolution in the other group of seed plants, the gymnosperms. In this work, we used high density gene-based linkage mapping to compare the karyotype structure of two families of conifers (the most abundant group of gymnosperms) separated around 290 million years ago: Pinaceae and Cupressaceae. We propose for the first time a model based on the fusion of 20 ancestral chromosomal blocks that may have shaped the modern karyotpes of Pinaceae (with n=12) and Cupressaceae (with n=11). The considerable difference in modern genome organization between these two lineages contrasts strongly with the remarkable level of synteny already reported within the Pinaceae. It also suggests a convergent evolutionary mechanism of chromosomal block shuffling that has shaped the genomes of the spermatophytes.

Conifer comparative genome analysis

Pär Ingvarsson on behalf of Amanda de La Torre,

Swedish University of Agricultural Sciences (Sweden)

Dissecting gymnosperm genomes and transcriptomes using the gymno-PLAZA comparative platform

Lieven Sterck[1], Zhen Li[1], Michiel Van Bel[1], Klaas Vandepoele[1] and Yves Van de Peer[1]

[1] Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Ghent, Belgium.

With the arrival of low-cost next-generation sequencing a host of new genomes and transcriptomes is being publicly released, providing significant opportunities for comparative and evolutionary studies in plants. Here we present gymno-PLAZA, the newest section of the PLAZA resource for plant comparative genomics, which provides a comprehensible research environment to aid researchers in the exploration of gene and genome information. The new platform addition features genome annotations for two members of the pinaceae as well as transcriptome data for several other conifers (6) and gymnosperms (3). The available data consists of structural and functional annotations of genes, homologous gene families, multiple sequence alignments, phylogenetic trees and inferred colinear regions within and between species. The web interface offers access to a wide variety of interactive tools (eg. the Integrative Orthology Viewer) and visualizations while the workbench gives researchers the opportunity to perform custom analyses on large user-defined datasets.

Genomic tools and genetic conservation

Stephen Cavers [Invited speaker]

NERC Centre for Ecology and Hydrology (UK)

A better understanding of the genetic basis of adaptive variation can help to improve management and use of forest genetic resources. Despite being the world's most widely distributed pine species, Scots pine (Pinus sylvestris L.) nevertheless faces severe population reduction, fragmentation and modification of native populations in Scotland. In addition, the remnant native populations face new challenges in novel pests and diseases and climate change, and a better understanding of their adaptation and evolutionary potential is called for in order to support the protection and restoration of the indigenous forest. In this part of its range, distinct adaptations to the northern maritime climate are apparent in general, but a combination of heterogeneous topography and strong longitudinal variation in climate has also produced highly localised adaptation. As a test case for examining the genetic basis of adaptation, these populations - arising from a large gene pool, with effective gene flow and negligible population structure - represent a valuable system. Using a combination of quantitative genetics, molecular markers and genomic tools, and in single-taxon and comparative analysis we place adaptive and genetic variation in Scots pine in context to evaluate the extent and dynamics of Scottish genetic resources.

Multi-trait genomic selection for breeding Eucalyptus hybrids - importance of additive and non-additive effects

Jean-Marc Bouvet [Invited speaker]

CIRAD French agricultural research and international cooperation organization (France)

Hybrids are broadly used in Eucalyptus breeding and accurate estimation of variance components is crucial for optimizing genetic gain. Genome-wide markers such as single nucleotide polymorphism may be used to explore models designed to assess the extent of additive and non-additive variance and their prediction accuracy for the genomic selection using single and multi-trait approaches.
In a first step, ten linear mixed models, involving pedigree- and marker-based relationship matrices among parents, were developed to estimate additive (A), dominance (D), and epistatic (AA, AD and DD) effects. Five complementary models, involving the gametic phase to estimate marker-based relationships among hybrid progenies, were developed to assess the same effects. The models were compared using tree height and 3303 SNP markers from 1130 cloned individuals obtained via controlled crosses of 13 Eucalyptus urophylla females with 9 Eucalyptus grandis males. AIC, variance ratios, asymptotic correlation matrices of estimates, goodness-of-fit, prediction accuracy and mean square error were used for the comparisons. The variance components and variance ratios differed according to the model. Models with a parent marker-based relationship matrix performed better than those that were pedigree-based, i.e. an absence of singularities, lower AIC, higher goodness-of-fit and accuracy and smaller mean square error. However, AD and DD variances were estimated with high standard errors. Using the same criteria, progeny gametic phase-based models performed better in fitting the observations and predicting genetic values. However, DD variance could not be separated from the dominance variance and null estimates were obtained for AA and AD effects. This study highlighted the advantages of progeny gametic phase-based model using genome wide information.
In a second step, using the progeny gametic phase-based model, the performance of multi-trait genomic selection was analysed using experimental data and simulations. With experimental approach, we used juvenile growth (height increment between 8 and 18 months), critical trait to avoid weed competition, and volume at mid-rotation age. Experimental data showed that multitrait- was slightly better than single trait genomic selection. This low difference can be explained by the very close heritabilities (h^2=0.36 for height increment and volume h^2=0.39). With simulation we considered two traits with contrasted heritabilities (h^2=0.10 and h^2=0.70) with additive and environmental correlation varying from 0.1 to 0.9. Compared to single trait genomic selection, the results showed that the prediction accuracy for a low-heritability trait could be significantly increased by multivariate genomic selection when a correlated high-heritability trait was available.

Keywords: relationship matrix, linear mixed model, variance components, G-BLUP, experimental data, simulations

Exploring the potential for genomic prediction in conifer breeding: a joint simulation study

João Paulo, Marco Bink, Joost van Heerwaarden, Laurent Bouffier, Jérôme Bartholome, Christophe Plomion, Harry Wu, Outi Savolainen Mikko Sillanpää Fikret Isik, Steve Lee, Leopoldo Sanchez, John Woolliams, Isobel StewartMarco Bink and Joost van Heerwaarden

Wageningen UR Biometrics (Netherlands) :

As genotyping costs have plummeted over the last decade, there is increasing use of molecular markers in plant and animal breeding programs. Based on early successes in livestock breeding, marker-based prediction of breeding values, or genomic prediction, has been heralded as a promising tool for increasing genetic gain. Genomic prediction could be of particular benefit in conifer breeding, where reliable phenotyping is costly and most economic traits take a long time to be expressed. On the other hand, the lack of linkage disequilibrium and low marker densities, caused by large population sizes and complex genomes respectively, may be detrimental to the success of genomic prediction in conifers.
Here, we present results from a joint study on the potential for genomic prediction in contrasting population types associated with different conifer breeding programs. Using computer simulations, we study the effect of population size, mating scheme (polymix and full sibs) and pedigree depth on the ability to predict true breeding values by a GBLUP approach.
We thereby evaluate the improvement achieved as the amount of relatedness information is increased from partial pedigree to genome-wide identity-by descent.
We find that in the case of polymix breeding, the largest gain in accuracy is caused by the additional pedigree information offered by molecular markers, providing scope for cost-effective ways of improving selection efficiency. Overall, the increase in accuracy achieved by genotypic information is relatively small, particularly in large breeding populations with shallow pedigrees, but may be worthwhile for smaller populations with a longer breeding history.

Integration of molecular tools into the maritime pine breeding program in France

Jérôme Bartholomé [1,2], Marjorie Vidal [1,2,3], Annie Raffin [1,2], Isabelle Lesur [1,2], Christophe Boury [1,2], Erwan Guichoux [1,2], Adline Delcamp [1,2], Joost van Heerwaarden [4], Marco Bink [4], Fikret Isik [5], Christophe Plomion [1,2], Laurent Bouffier [1,2*]

[1] INRA, UMR1202 BIOGECO, F-33610 Cestas, France
[2] Univ. Bordeaux, BIOGECO, UMR 1202, F-33600 Pessac, France
[3] FCBA, Biotechnology and Advanced Silviculture Department, Genetics & Biotechnology team, 33610 Cestas, France
[4] Biometris, Wageningen University and Research Centre, NL-6700 AC Wageningen, Netherlands
[5] North Carolina State University, Department of Forestry and Environmental Resources, Raleigh, NC, USA

Maritime pine breeding in France started in the 1960ies with the selection of 635 founders (called G0 trees) in forest stands located in the Aquitaine region. A classical recurrent selection strategy to improve growth and wood quality is currently implemented using both progeny testing for genetic evaluation and bi-parental mating for selection. In this presentation we detail the progress of the integration of molecular tools into the maritime pine breeding program. From the verification of genetic identity to genomic selection approaches via polymix breeding strategy, we will present examples at different level of implementation. SNP arrays for DNA fingerprinting were developed for forest managers and breeders. These tools were successfully used in a study case of polymix breeding strategy where an increase of accuracy for genetic parameters and a better estimation of breeding values for the progeny were obtained. In addition, two proof-of-concept studies of genomic selection were established and have shown medium to high accuracy for the two main traits of the breeding program: growth and stem straightness.

Back to nature: candidate genes, population genomics and prediction of maladaptation in natural populations

Santiago C. González-Martínez[1,2] [Invited speaker], Ricardo Alía[3], Katharina Budde[4], Andrew J. Eckert[5], Delphine Grivet[3], Myriam Heuertz[1,2], Juan-Pablo Jaramillo-Correa[6], Maria Mayol[7], Christophe Plomion[1,2], Miquel Riba[7], Isabel Rodríguez-Quilón[3], Giovanni G. Vendramin[8], Natalia Vizcaíno-Palomar[3,9] (authors in alphabetical order, except presenting author)

[1]UMR1202 BIOGECO: Biodiversity, Genes & Communities, INRA, Cestas, France;
[2]UMR1202 BIOGECO: Biodiversity, Genes & Communities, University of Bordeaux, Talence, France;
[3]Forest Research Centre, INIA, Madrid, Spain;
[4]Forest Research Centre, University of Copenhagen, Frederiksberg C, Denmark; [5]Virginia Commonwealth University, Richmond, USA;
[6]UNAM, Institute of Ecology, México City, Mexico;
[7]CREAF, Cerdanyola del Vallés, Spain;
[8]National Research Council, Institute of Biosciences and Bioresources, Sesto Fiorentino, Italy;
[9] Forest Ecology and Restoration Group, University of Alcalá , Alcalá de Henares, Spain.

Population and ecological genomics hold promise to identify relevant drivers of genetic adaptation to climate, as well as the relevant genes and gene networks associated with this process. Most important variation for adaptation is expected to be polygenic. However, most research to date has been based on single loci. Moreover, there is an urgent need to establish field experiments that help connecting molecular variation with fitness surrogates in a wide variety of natural environments. In this talk, we first provide a conceptual framework for population genomic studies to better understand the genetic bases of adaptation, in particular in reference to recent progress in human genetics. We also highlight the difference between “mutational” and “allelic” variation, which may underlie the highly contrasted results obtained from QTL and association genetics studies in terms of allelic effects, as well as explain the higher than expected success of candidate gene studies (in comparison with larger studies based on less well characterized markers). Second, we develop different case studies in European conifers, providing evidence on molecular adaptation to climate in two widespread species with highly contrasted population structure, the maritime pine (*Pinus pinaster* Aiton) and the English yew (*Taxus baccata* L.). In both cases, common gardens and quantitative genetic analyses of fitness-related traits were fundamental to either identify selection drivers (e.g. continentality in yew) or to validate genotype-environment associations (in maritime pine) by associating allelic make-up with fitness under extreme environmental conditions. For maritime pine, we also present preliminary results on the relevance of polygenic adaptation to climate, and on how studies that consider gene interactions may overcome previous limitations to identify relevant adaptive variation in this species. Finally, current studies at local spatial scales in different conifers and their comparison with rangewide adaptation patterns stress the need to explore different geographical scales of genetic adaptation.

Sequence to Phenotype: Allocation of Resources

John M Hickey[1,*], R Chris Gaynor[1], Ian J Mackay[2], Gregor Gorjanc[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Research Centre, Midlothian EH25 9RG, UK
[2]John Bingham Laboratory, NIAB, Huntingdon Road, Cambridge, CB3 0LE, UK
* John.Hickey@roslin.ed.ac.uk

Background
Genomic selection is increasingly valued within the plant breeding community. To implement genomic selection large investments are needed in genomic data (markers and or sequence) and phenotypic data on which to train prediction equations. Choices about distributing these resources affect the return on investment

Results
A simulation was conducted which evaluated the long term benefit of three alternative breeding program designs: (i) a classical plant breeding program design; (ii) a minor modification to the classical design in which genomic prediction was used to increase the accuracy of preliminary yield trials; and (iii) a complete reorganization of the breeding program into a population improvement component driven by genomic selection and a product development component that was similar to i.

Conclusions
The different breeding program designs gave different returns on investment. Complete reorganization of plant breeding programs into population improvement components driven by genomic selection and product development components was promising but its benefit was affected by costs.

Economic evaluation of new breeding strategies in Maritime pine (*Pinus pinaster*)

Luc Harvengt

FCBA Institut Technologique Forêt Cellulose Bois-construction Ameublement (France)

Three alternative forward breeding strategies (FBS, i.e. polymix breeding with or without genotyping use for pedigree reconstruction and Genomic Selection, GS) have been compared to the backward breeding strategy currently used by the French Maritime Pine Breeding Cooperative (GIS Pin Maritime du Futur= GPMF). The GPMF breeders (INRA and FCBA staff) agreed on achievable genetic gain within a single generation for the four breeding strategies to be studied in the case of three breeding targets i.e. improving (1) tree volume alone, (2) tree straightness alone or (3) combination of the two parameters. The corresponding breeding activities and seed orchard establishment operations were detailed with their time schedule and costs. Then corresponding tree growth and silviculture were simulated again with time scheduled costs and incomes. The increased growth speed determinate a very significant shortening of the optimal cropping cycle. A full financial benefit cost analysis was performed on these bases, revealing the relative difference of the 12 strategies x targets scenarios. Sensitivity analyses were performed with wood price (per wood grade). While the additional costs of all the FBS were found to have negligible impact on the seedling price but a significant positive impact on forest owner benefit. Variations in wood price had a strong impact, total wood quantity being by far the most efficient breeding trait to target in order to get a maximum financial return in the current wood market condition.

Designing core collections from natural range distributions

Giovanni Vendramin

Outi Savolainen[1], Jaakko Tyrmi[1], Tanja Pyhäjärvi[1], Catherine Bastien[2], Vanina Guerin[2], M. Teresa Cervera[3], M. Ángeles Guevara[3], Giovanni Giuseppe Vendramin[4]

[1] University of Oulu, Oulu, Finland
[2] INRA, Orleans, France
[3] INIA-CIFOR, Madrid, Spain
[4] IBBR-CNR, Firenze, Italy

Core collections represent strategic repositories of gene pools for key species, and are often a baseline for advanced breeding. They must cover efficiently both distinct demographic units and the adaptive genetic structure at the natural range of those key species. The ability to genotype many neutral loci provides much better estimates of the patterns of reproductive isolation and demographic history of populations to address demographic distinctiveness. Genomic approaches for studying functional genes have been used to evaluate the amount of adaptive divergence among populations required in the assessment of adaptive genetic structure. We used available SSR markers to examine population differentiation. We used exome capture to discover and measure sequence variation of targeted areas of the Scots pine exome. We had earlies used baits designed based on the Pinus taeda genome, and now probes were designed based on the P. sylvestris transcriptome. We present and discuss some results on European wide sampling, on numbers of SNPs, patterns of genetic differentiation and linkage disequilibrium.

RAD sequencing in Sitka Spruce for Genomic Selection

John Woolliams

University of Edinburgh - Roslin Institute (UK)

General discusion and wrap-up

Expression patterns of drought-related genes in *Pinus pinaster*

Dolores Abarca[1], Inmaculada Hernández[1], Alicia del Amo[1], Elena Carneros[1], Alberto Pizarro[1], Amanda R. De La Torre[2,3], Yao-Cheng Lin[4], Par K. Ingvarsson[2], Concepción Ávila[5], Francisco Cánovas[5], Ángeles Guevara[6], Nuria de María[6], Mª Dolores Vélez[6], María Teresa Cervera[6], Carmen Díaz-Sala[1]

[1] Departamento de Ciencias de la Vida, Universidad de Alcalá. Ctra. de Barcelona Km 33,6, 28805 Alcalá de Henares, Madrid, Spain
[2] Umeå Plant Science Centre, SE-90187, Umeå, Sweden
[3] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90187 Umeå, Sweden
[4] Department of Plant Systems Biology (VIB) and Department of Plant Biotechnology and Bioinformatics (Ghent University), Technologiepark 927, 9052 Ghent, Belgium
[5] Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Campus Universitario de Teatinos, Universidad de Málaga, 29071 Málaga, Spain
[6] Departamento de Ecología y Genética Forestal, INIA-CIFOR, Ctra. de La Coruña Km 7.5, 28040 Madrid, Spain

Plants are prepared to respond to environmental fluctuations that affect their development and physiology. Extreme unfavorable conditions such as low water availability can have a strong impact in fitness and productivity. Responses to drought have been characterized mostly in annual plant species, and key regulatory genes have been successfully used to improve tolerance. However, the specific mechanisms that allow long-living species, such as conifers, to survive extensive periods of water stress are not clearly defined. A combination of short-term and long-term responses seems to be essential for adaptation to environments featuring annual periods of drought.
A sequential approach to analyze responses to water stress in a group of 330 pine genes led to the identification of specific members of gene families that responded selectively to osmotic stress. Comparative genomic analysis showed conifer-specific expansions in gene families of five stress-related candidate genes. All of them exhibited differential expression in Pinus pinaster clones with contrasting drought tolerance phenotypes. In order to analyze the involvement of these genes in organ-specific pathways associated with the response to water stress, constitutive transcript profiles of these genes, and changes in their mRNA levels after long-term and short-term drought treatments, have been analyzed in organs of plants and seedlings, respectively. The transcript profile of these genes was also analyzed in the presence of abscisic acid (ABA), a key plant stress-signaling hormone. Changes in the DNA methylation patterns are under analysis, in order to identify tissue-specific pathways regulated by epigenetic mechanisms that might be associated with the response to water stress.

Acknowledgements
The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement nº 289841.

Phenotypic analysis of transgenic *Pinus pinaster* lines overexpressing MYB5

Dolores Abarca[1], Elena Carneros[1], Inmaculada Hernández[1], Alberto Pizarro[1], Jean-François Trontin[2], Carmen Díaz-Sala[1]

[1]Departamento de Ciencias de la Vida, Universidad de Alcalá. Ctra. de Barcelona Km 33.6, 28805 Alcalá de Henares, Madrid, Spain
[2]FCBA, Biotechnology and Advanced Forestry Department, Genetics & Biotechnology Group, 71, Route d'Arcachon, Pierroton, 33610 Cestas, France.

Functional analysis of genes potentially involved in processes, such as lignin biosynthesis, with physiological, ecological and economic interest, is an essential step in the selection and development of suitable biotechnological tools to improve plant properties. In order to gain insight into the possible function of Pinus pinaster MYB5, a gene encoding a transcription factor potentially involved in the regulation of lignin biosynthesis, overexpression transgenic lines were obtained using a somatic embryogenesis approach (Trontin et al., 2013, Proc IUFRO Somatic Embryogenesis and Other Vegetative Propagation Technologies, Brno, Czech Republic, pp 184-187). For phenotypic analyses, embryogenesis and germination protocols were optimized and an in vitro shoot multiplication system was developed to obtain multiple cuttings from individual transgenic lines. Both embryogenic masses and cuttings were exposed to UV light and osmotic or water stress to assess the response of MYB5 and other related genes to the treatments.
Embryogenic lines showed no visible symptoms after the treatments; however, changes in MYB5 expression were observed. In addition, changes in the level of expression of other lignin biosynthesis-related genes were detected in transgenic lines overexpressing MYB5. Treated cuttings did show detectable symptoms, but both overexpression and gene responses to the treatments were strongly reduced, suggesting a dominant effect of regulatory circuits associated to development.

Acknowledgements
The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement nº 289841.

Pinus pinaster smallRNA sequencing data analysis pipeline

Andreas Bohn[1,2], Bruno Costa[1], Andreia Rodrigues[1,2], Inês Chaves[1,2], Célia Miguel[1,2*]

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. República, 2780-157 Oeiras, Portugal

The analysis of smallRNAs (sRNA) by next generation sequencing (NGS) is a powerful tool to discover novel molecular regulation pathways underlying important biological processes in a wide range of organisms, including forest trees.
The identification of different sRNA classes, analysis of their expression profiles and prediction of their target genes in a given dataset requires the sequential application of several data processing and analysis tools. We developed a pipeline to allow the automated analysis of sRNA datasets from multiple tissues, developmental stages and growth conditions of P. pinaster.
The pipeline was built around a core of several modules from the publicly available University of East Anglia small RNA workbench (UEA sRNA WB, [1]), deployed to a dedicated Linux server to be used via the command line interface, with UNIX shell scripts performing basic data input and output operations. Filtered sequences were aligned to the genome of P.taeda [2]. Further alignment of the genome-aligned reads with mirBase v21 [3] yielded the conserved miRNAs. The corresponding sRNA WB tools for the prediction of novel miRNAs and ta-siRNAs and corresponding targets were subsequently applied. For target prediction, the available annotated transcriptome of P.pinaster [4] was applied.
We analyzed datasets from 38 sRNA sequence libraries using the implemented pipeline. The obtained results will be presented and, given their general plausibility we conclude that this pipeline provides a useful tool for further exploring the role of small RNAs in P.pinaster development and adaptation.

Acknowledgements
FCT through grants SFRH/BD/79779/2011 (ASR) and project EXCL/EEI-ESS/0257/2012, and EU for support through project PROCOGEN nº 289841

References
1. Stocks M et al (2012) Bioinformatics 28:2059-2061
2. Neale D et al (2014) Genome Biology 15:59-59
3. The miRBase Sequence Database, Release 21. ftp://mirbase.org/pub/mirbase/21
4. Canales J et al (2014) Plant Biotechnol J 12: 286-299"

Transcriptomic analysis of *Pinus canariensis* during the resprouting process in response to mechanical wounding

Carmen Collada and Álvaro Soto

GENFOR, Grupo de Investigación en Genética y Fisiología Forestal. ETSI Montes. Universidad Politécnica de Madrid.

Resprouting is a relevant feature that allows plants to recover after grazing, wildfire or other disturbances. Pinus canariensis is the only conifer in the Old World able to resprout as response to damage. In order to analyze gene expression profiling during lateral shoot resprouting, 5 year-old Canary Island pines grown in experimental plot were wounded and induced resprouts were harvested at three times during their development: i) immediate (R0), ii) intermediate (R1), and iii) late (R2). Transcriptomic analysis of the resprouting process was performed using a microarray containing 20,000 selected unigenes involved in Pinus canariensis cambial activity. Over the period of response, more than 500 unigenes modified significantly their level of transcripts and were considered Differentially Expressed Genes (DEGs). Genes involved carbohydrates synthesis and mobilization during cell wall formation, cell wall weakening and lignification process were found in this group. Overexpression in R0 and downregulation during R1 and R2 were found for genes presumably related to response to wound and biotic stress, such as antimicrobial peptide 1-like, disease resistance response protein 206-like, or pathogenesis-related protein PR-4b-like. We found significant increasing transcription throughout the resprouting process, for DEGs encoding for transcription factors belonging to GROWTH-REGULATING FACTOR (GRF), FLOWERING-PROMOTING FACTOR (FPF), LEAFY (LFY) and YABBY (YAB) families, and the HOMEODOMAIN LEUCINE ZIPPER Class IV (HD-ZIP IV) subfamily. Homology of these genes suggests a common regulation of the resprouting process and constitutive development of inflorescences and lateral sprouts.

Novel and known microRNAs identified in vascular tissues in Scots pine and Maritime pine

Ana Carvalho[1], Clara Graça[2], Victor Carocha[3], Susana Pêra[2], José Lima-Brito[1], Gregoire Le Provost[4], Jorge A. P. Paiva[2,5]

[1] Centro de Genómica e Biotecnologia Agrária (CGBA), Universidade De Trás-Os-Montes E Alto Douro, 5001-801 Vila Real, Portugal (anacarvalho@mail.com)
[2] Instituto De Biologia Experimental E Tecnológica (ibet), Apartado 12, 2780-901 Oeiras, Portugal (cgraca@ibet.pt)
[3] Instituto De Tecnologia Química e Biológica, Universidade Nova De Lisboa, Av. Da República, 2780-157 Oeiras, Portugal (carocha@itqb.unl.pt)
[4] UMR Biodiversité Gènes et Communautés INRA 69 route d'Arcachon 33612 CESTAS Cedex (leprovost@pierroton.inra.fr)
[5] Department of Integrative Plant Biology, IGR PAN, Strzeszynka 34, 60'479 POZNAN, Poland (jpai@irg.poznan.pl; jorgep@ibet.pt)"

Among conifers, Pines have high economic importance mainly due to wood and resin production. In the last decade, several transcriptomic and proteomic resources were made available providing opportunities to identify major molecular players involved in xylogenesis and biotic and abiotic responses. Nevertheless, the understanding of post-transcriptional regulation mediated by miRNA in Pines remains scarce. MicroRNAs are small non-coding RNAs (21-24bp) that act by down-regulating mRNA expression either by cleavage or by translational repression, through direct base-pairing to target sites. Genome-wide identification of miRNAs and the perception of the impact of their regulatory roles on plant development and stress responses is susceptible to benefit the definition of strategies for wood quality improvement or stress response strategies.Here, we will present the first results on the identification of miRNA present in vascular tissues (developing xylem and phloem) and needle epidermis of two pine species (P. pinaster and P. sylvestris) of major economic and ecological importance in European forest. High-throughput sequencing of the 5 small RNAs libraries generated a total of 6,4M raw reads, from which 5.6M are considered mappable, with a size modal distribution (21nt, 32% of the reads). 5,832 miRNA candidate loci, including 44 known miRNAs already identified in other species (1,3 M reads) and 1,291 new high-confidence miRNA candidates (9,9M reads). The majority of the known miRNAs was already identified in P. taeda and P. densata. Seventy four miRNA sequences with highest abundance (number of reads higher than the average of 1059 reads) were selected for testing their presence in the several sampled tissues and genomes. Additionally, degradome sequencing analysis allowed us to detect 357, 312, 534, 1790 and 428 interactions between target genes and putative miRNAs for P. pinaster and P. sylvestris. The genomic resources presented here stand out with great relevance for the understanding of post-transcriptional regulation underlying the development of the vascular tissues and stress responses in conifers.

Acknowledgements
The authors thank theEuropean project FP7-KBBE-2011-5/289841 (PROCOGEN). AC and VC thank FCT for SFRH/BPD/68932/2010 and SFRH/BD/72982/2010 Grants, respectively, co-financed by FSE/POPH-QREN. CG and SP thank the research grants in the frame of PROCOGEN. JAPP acknowledges the research contract in the frame of the EU BIO-TALENT (The Creation of the Department of Integrative Plant Biology) project submitted under FP7-ERAChairs-Pilot Call-2013 (Grant agreement nº621321.

Improving biomass for bioenergy and bioproducts in Italy: the case of Franco Alasia Vivai

Francesco Fabbrini[1*], Omar Alasia[1], Maurizio Sabatti[2], Alessandro Merlone[1], Gail Taylor[3], Antoine Harfouche[2], Franco Alasia[1]

[1] Alasia Franco Vivai s.s., loc. Solerette 5/a, 12038 Savigliano (CN), Italy,
[2] Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, via S. Camillo de Lellis snc, 01100 Viterbo, Italy,
[3] Centre for Biological Sciences, University of Southampton, UK
* Email: r&d@alasiafranco.it

Ensuring increased and sustainable biomass production is critical for European countries. Short-rotation coppice (SRC) culture have a great potential to increase biomass supply for bioenergy and bio-based products in Europe. Alasia Franco Vivai (AFV) is one of the biggest EU company in the production of plant material for energetic woody crops. To meet the market needs, AFV collect genotypes and develop new germplasm of four of the most used and promising tree species in Europe: Poplar, Willow, Arundo, and Miscanthus. The business development pipeline for the two strategic crops, *Populus* and *Arundo*, is very active with the company looking to expand to additional territories. Furthermore, to strengthen commercial and research activities, AFV has explored potential commercial opportunities (Greenwood resources, USA; International Paper, USA; ENCE, Spain), has established research collaboration with public entities (CNR, University of Tuscia, University of Pisa, University of Pavia), and has been involved in EU projects (POPYOMICS, BENWOOD, NOVELTREE and WATBIO). In order to make SRC system a sustainable opportunity, the selection of new high yielding and site-specific genotypes, along with the evaluation of phenotypic plasticity and genotype × site interaction, are of paramount importance. The overall purpose of WATBIO (developing drought-tolerant biomass crops for Europe - http://www.watbio.eu/) is to raise the economic and environmental performance of biomass crops grown on drought-stressed marginal lands by improving the efficiency of plant breeding. In the frame of this project, AFV plays a major role in shaping the project from a commercial exploitation perspective in Europe and plays a major role in the delivery of impact from relevant exploitable outputs. Furthermore, AFV has actively contributed by establishing and managing field trials activities on *Populus nigra* and *Arundo donax* in Italy, by supplying putative ecotypes of *A. donax* collected across Europe for studying genetic variability and by participating to the field data collection. Altogether, efforts of this research will focus on delivering new germplasm of non-food biomass for second-generation bioenergy, while protecting already stretched water resources.

Key words: Energy crops, breeding, biomass, bioenergy, bio-based products.

Genome discovery of deep frost-tolerant eucalyptus for breeding and molecular ecology

Luc Harvengt[1], Hélène San Clemente[2], Jean-Marc Gion[3], Christophe Klopp[4], Hélène Berges[5], Brad Potts[6], René Vaillancourt[6], Christiane Marque[2], Chantal Teulières[2] and Jacqueline Grima-Pettenati[2]

[1] Genetics and Biotechnology team, Biotech and Advanced Forestry Department, FCBA tech institute, Campus Recherche Forêt-Bois de Pierroton, 71 route d'Arcachon, 33610 Cestas
[2] Laboratoire de Recherches en Sciences Végétales, LRSV UMR CNRS/ Université Toulouse III, 24, chemin de Borde Rouge, Auzeville, BP42617, F-31326 Castanet Tolosan, France
[3] CIRAD UMR AGAP, GS team, Localized at INRA UMR BIOGECO 1202, 69 route d'Arcachon, F-33612 CESTAS Cedex, FRANCE
[4] Plate-forme Genotoul Bioinfo, INRA Toulouse, 24 Chemin de Borde Rouge – Auzeville CS 52627, F-31326 Castanet Tolosan cedex, France
[5] French Plant Genomic Resource Center, INRA – CNRGV, 24 Chemin de Borde Rouge CS 52627, F-31326 Castanet Tolosan, FRANCE
[6] School of Biological Sciences and National Centre for Future Forest Industries, University of Tasmania, Private Bag 55 Hobart 7001 Tas, Australia
Contact e-mail: luc.harvengt@fcba.fr; grima@lrsv.ups-tlse.fr

In contrast to some instinctive expectations, frost tolerance becomes increasingly a concern in areas experiencing rapid climate changes. Eucalypts leading breeders as well as landowners are seeking for cold-tolerant germplasm for expanding plantations towards colder areas as well as to secure wood and biomass production at places already subjected to severe sudden frosts. From an ecological point of view, eucalypts constitute with oaks another model system of species complexes with clinal variations among ecotypes of huge interest for understanding forest tree adaptation to the environment and climate change and ecosystem/community genetics (Kremer *et al.* 2014, Whitham *et al.* 2006, Crutsinger 2015). FCBA has been breeding frost-tolerant eucalyptus for about 50 years, focusing on E gunnii and E dalrympleana. FCBA varieties have been used for decades as models for genomic and physiological investigations by the Plant Science Laboratory (LRSV, in Toulouse, team 2), leading to significant results regarding the dissection of molecular processes related to cold response and wood formation. Remarkably, several key genes involved in the biosynthesis of lignin and the regulation of the secondary cell wall formation were first cloned in *E. gunnii*. The earliest public releases of large sets of ESTs in *Eucalyptus* were from *E. gunnii*. Recently, the first genetic map and some *de novo* genome sequencing has been performed for one reference *E gunnii* genotype. We propose to go further, sequencing also a *E dalrympleana* whole genome and resequencing a number of individuals from natural populations as well as the breeding population. Comparative studies with the E. grandis reference genome will improve our understanding of the molecular bases, evolutionary history and genomic organization of frost tolerance and wood production in *Eucalyptus*. In addition, these genome sequences will constitute a highly valuable resource to further dissect the gene flow between and within species of the *Eucalyptus* species complex of the Tasmania central plateau and shed a new light on the associated ecosystems.

Crutsinger G. M. (2015). New Phytologist: Doi: 10.1111/nph.13537
Kremer A., Potts B. M. and Delzon S. (2014). Functional ecology 28: 22-36. Doi: 10.1111/1365-2435.12169
Whitham T. G., et al (2006). Nature Reviews Genetics Doi:10.1038/nrg1877

Conserved Orthologous Sets across Gymnosperms as Effective Markers for Phylogenomics in the Seed Plants

Zhen Li[1,2], Amanda R. De La Torre[3,4], Lieven Sterck[1,2], Francisco M. Canovas[6], Concepcion Avila[6], Pär K. Ingvarsson[3,5], Yves Van de Peer[1,2,7]

[1] Department of Plant Systems Biology, VIB, Ghent B-9052, Belgium
[2] Department of Plant Biotechnology and Bioinformatics, Ghent university, Technologiepark 927, 9052 Ghent, Belgium
[3] Umeå Plant Science Centre, SE-90187, Umeå, Sweden
[4] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90187 Umeå, Sweden
[5] Department of Ecology and Environmental Science, Umeå University, Linneaus väg 6, SE-901 87 Umeå, Sweden
[6] Departamento de Biologia Molecular y Bioquimica, Facultad de Ciencias, Instituto Andaluz de Biotecnologia, Universidad de Malaga, Campus Universitario de Teatinos s/n, 29071 Malaga, Spain
[7] Genomics Research Institute, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa.

Over the past few years, numerous genomic and transcriptomic resources have accumulated in gymnosperms, the genomes of which are difficult to assemble because of their tremendously larger genome sizes comparing with other eukaryotic genomes. Although most of the current sequences are from transcriptomes, comparative analyses based on transcriptomic data can provide evolutionary insights before enough gymnosperm genomes become available. It can also accelerate comparative genetic mapping between different gymnosperm species, especially between conifers, which are of primary interest for quantitative genetics and comparative genomics. To cross the limits that only a few genetic markers are available in gymnosperms, we constructed 3,072 conserved orthologus set (COS) markers in 31 gymnosperms from 11,152 low-copy number gene families from six Pinaceae species, which either have genomes (*Picea abies* and *Pinus taeda*), high quality transcriptomes (*Picea glauca*, *Picea sitchensis*), or deep sequenced transcriptomes (generated by ProCoGen project for *Pinus pinaster* and *Pinus sylvestris*). Using the relative high quality transcriptomes promised us to find 2,539 COS markers in angiosperms from PLAZA 3.0 and 1,468 COS markers in the seed plants. Moreover, 42 high quality phylogenetic markers of nuclear genes for the seed plants were identified to resolve position of Gnetales and relationships in Pinales.

Analysis of differentially regulated expressed sequences and microRNAs in *Pinus sylvestris* in response to methyl jasmonate treatment

Elza Rauda, Baiba Krivmane, Ilze Snepste, Angelika Voronova, Vilnis Skipars, Ilze Veinberga

Latvian State Forest Research Institute "Silava"

Plants have developed a range of epigenetic effects to deal with biotic and abiotic stress, including DNA methylation, histone modification and non-coding RNA (including microRNA), which influence gene expression and regulation. The importance of the role of microRNAs (miRNAs) in post-transcriptional gene regulation is increasingly being recognised.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), are plant signalling molecules that affect plant growth and gene expression. Methyl jasmonate is plant hormone synthesized from linoleic acid and plays a role in plant responses to abiotic and biotic stresses, including wound response and plant defence. MeJa has also been implicated in induced resistance responses in plants.

The aims of this study were to identify and characterise RNA sequences differentially expressed in response to methyl jasmonate treatment in Scots pine. Both long and short RNA libraries were sequenced using the IonTorrent PGM platform. Expressed sequences, were characterised and differential expression of genes and miRNAs was investigated. This enabled identification of microRNAs in Scots pine, and will assist in the elucidation of the role of miRNAs in defence responses. The results will allow investigation of changes in miRNA expression levels under stress conditions and identification of potential target genes.

The microRNA transcriptome of the developing embryo of *Pinus pinaster*

Andreia S. Rodrigues[1,2*], Inês Chaves[1,2], Susana Lopes[1,2], Yao-Cheng Li[3], Yves Van de Peer[3], Célia Miguel[1,2]

[1] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[2] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. República, 2780-157 Oeiras, Portugal
[3] Department of Plant Systems Biology (VIB) and Department of Plant Biotechnology and Bioinformatics (Ghent University), Ghent, Belgium

Transcriptomic analysis of P. pinaster zygotic embryogenesis (ZE) highlighted several epigenetic regulation mechanisms [1] and showed that functions related to smallRNA (sRNA) pathways appear differentially regulated across embryo development with a prevalence of microRNA (miRNA) functions in mid to late embryogenesis. In this work, we analyzed the sRNA transcriptome for the same ZE developmental stages previously studied.
SmallRNA libraries were prepared from pools of embryos at 5 developmental stages [2] and sequenced. Bioinformatic analysis was done using an in-house pipeline (https://github.com/forestbiotech-lab/sRNA-workflow) and CLC Genomics Workbench [3]. Different methodologies, including RT-PCR, RT-qPCR and Northen blotting, have been performed to validate a set of conserved and putative novel miRNAs. miRNA target genes have been predicted against the transcriptome of P. pinaster [4], and degradome sequencing was performed for validation of targets.
Unlike suggested in previous reports on sRNA analysis in gymnosperms, pine shows a high abundance of the 24-nt sRNAs in embryo tissues; this is also the most diverse size class of sRNAs found in the ZE libraries. The number of conserved miRNAs is within the range found in other plant species and represents a small fraction of the pine sRNA transcriptome. MIR166 was the most expressed conserved MIR family and the one comprising the largest number of isoforms. Degradome results suggest that putative novel miRNAs generate most of the cleaved target genes found across ZE, which are also the most abundant ones.

Acknowledgements
FCT through grant SFRH/BD/79779/2011 (ASR) and EU through project PROCOGEN nº 289841. I. Carrasquinho and A. Aguiar (INIAV) for provision of plant material.

References
1. de Vega-Bartol J et al (2013) BMC Plant Biology 13:123
2. Gonçalves S et al (2005) Planta 222:556-563
3. CLC Genomics Workbench, CLC bio [http://www.clcbio.com]
4. Canales J et al (2014) Plant BiotechnolJ 12:286-299

Towards functional genomics of transcription factor genes associated to growth and wood formation in maritime pine

Jean-François Trontin[1], Concepción Avila[2], Sandrine Debille[1], Fernando de la Torre[2], Jorge El-Azaz[2], Belén Pascual[2], Francis Canlet[1], Caroline Teyssier[3], Boizot N[3], Le Metté C[3], Lesage-Descauses M-C[3], Denilson da Silva Perez[4], Rafael Cañas[2], Grégoire Le Provost[5], Christophe Plomion[5], Luc Harvengt[1], Philippe Label[6], Marie-Anne Lelu-Walter[3], Francisco Cánovas[2]

[1] FCBA, Pôle Biotechnologie et Sylviculture Avancée, 71 route d'Arcachon, Pierroton, 33610 Cestas, France
[2] Universidad de Málaga, Dept. Biología Molecular y Bioquímica, Facultad de Ciencias e Instituto Andaluz de Biotecnología, Campus de Teatinos S/N, 29071 Málaga, Spain
[3] INRA, UR 0588 Unité Amélioration, Génétique et Physiologie Forestières, 2163 avenue de la Pomme de Pin, CS 4001, Ardon, 45075 Orléans Cedex 2, France
[4] FCBA, Pôle IntechFibres, Domaine Universitaire, CS 90251, 38044 Grenoble Cedex, France
[5] INRA-Université de Bordeaux, UMR 1202 BIOGECO, 69 route d'Arcachon, Pierroton, 33612 Cestas, France
[6] INRA-Université Blaise Pascal, UMR A547 PIAF, Les Cézeaux, 24 Avenue des Landais, 63177 Aubière cedex, France

Maritime pine is a major forest tree in southern Europe for sustainable delivery of bio-based products through advanced plantation forestry of improved varieties. Early selection is needed to reduce breeding cycle and accelerate variety deployment in the context of climate change. Both gene(s) and genome-wide information should provide opportunities for predictive, marker-assisted selection. Reverse genetics, defined as ectopic expression or silencing of candidate genes can be useful for functional dissection of traits of interest. Functional genomics of 9 transcription factor (TF) genes associated to growth and wood formation (*MYB1,8,14,20,23, DOF5, MADBOX4, NACx, NACataf*) was initiated in maritime pine through genetic transformation of embryogenic tissue. Twelve TF constructs designed for constitutive overexpression (OE) or silencing (RNAi) were studied: 6 constructs (batch 1) from previous projects (*MYB1*-RNAi, *MYB8*-OE/RNAi, *MYB14*-RNAi, *DOF5*-OE/RNAi) as well as 3 constructs for putative gene targets of *MYB* (*CAD*-RNAi) and *DOF5* (*GS1a*-OE, *GS2*-OE); and 6 constructs (batch 2) obtained during ProCoGen (*MYB23*-OE, *MYB20*-OE, *MADBOX4*-OE, *NACx*-OE/RNAi, *NACataf*-OE).

Phosphinothricin-resistant lines could be cryopreserved for all but one (*MYB20-OE*) constructs. Transformation rate was estimated in the range 9.6-22.0 (OE) or 0.4-18.0 (RNAi) transgenic lines per gram embryogenic tissue (batch 1). Somatic embryos were obtained from 1-3 lines per construct (25 lines in total) and successfully converted to plants (batch 1). Acclimatization rates were similar to controls and transgenic plants were confirmed for most lines. Putative adverse effect of *MYB14*-RNAi, *DOF5*-RNAi, and *MYB8*-OE on transformation rate was observed. *DOF5*-RNAi apparently stimulated germination rate. Plant growth data and morphology are available for constructs from batch 1 after up to 12 (*MYB1*-RNAi, *MYB8*-OE/RNAi, *DOF5*-OE/RNAi, *GS2*-OE, *GS1a*-OE) or 42 months (*MYB14*-RNAi, *CAD*-RNAi) growth. Both transgene copy number and targeted gene expression data are available from transgenic plants obtained from *MYB14*-RNAi and *CAD*-RNAi lines. Transgenics and controls from batch 1 were sampled at age 16 (*MYB1*-RNAi, *MYB8*-OE/RNAi, *DOF5*-OE/RNAi) or 70 months (*MYB14*-RNAi, *CAD*-RNAi). Molecular characterization of *MYB1,8,14* and *DOF5* plant material has been performed by qPCR and transcriptomic analysis using microarray is underway. Wood analyses have been initiated for *MYB14-* and *CAD-RNAi* plants (in progress).

This work received funding from the European Community's Seventh Framework Programme (FP7/2007-2013, Grant Agreement N°289841-PROCOGEN).

Genome-wide identification and profiling of novel and conserved miRNAs involved in formation of an epigenetic memory during embryogenesis in Norway spruce

Igor A. Yakovlev, Adam Vivian-Smith, Carl Gunnar Fossdal

Norwegian Institute for Bioeconomy, PO box 115, 1431, Ås, Norway"

Epigenetic memory in Norway spruce permanently affect the timing of bud burst and bud set, vitally important adaptive traits, in this long-lived forest species. MicroRNAs (miRNAs), a class of small noncoding RNA molecules have recently drawn attention for their prominent role in development and epigenetic regulations.
We prepared 18 small RNA libraries from embryogenic tissues of two individuals at three stages of maturation grown up in vitro at three culturing temperatures (18, 23 and 28°C). Obtained libraries were sequenced in duplicate on PGM™ (Ion Torrent™) system and analyzed using CLC genomic workbench.
In this study, we report the identification of more than 1100 novel and conserved miRNAs in Norway spruce derived from 1050 precursors. We found high amount of isomiRs and high redundancy of putative miRNA genes in released Norway spruce genome v1. Based on identified miRNAs we studied their expression patterns in dependence on the temperature prevailing during SE growth and leading to establishing of epigenetic marks. Distinct temperature dependent expression patterns were determined for most of analyzed miRNAs. miRNAs are targeting the large amounts of spruce genes with a wide range of functions, including genes involved in epigenetic regulation.

Genome-wide association studies

Marco Bink

Genomic selection

Leopoldo Sanchez

Using genomics tools to study local adaptation

Outi Savolainen

University of Oulu (Finland):

Transcriptomic profiles to study development and adaptation in maritime pine

Concepción Ávila, Rafael A. Cañas, Fernando de la Torre, Mª Belén Pascual, Francisco M. Cánovas

Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos s/n 29071 Málaga Spain

Maritime pine is a conifer species with great economic and environmental value that is widely distributed in the south-western area of the Mediterranean region. It has high phenotypic plasticity accompanied by high tolerance to abiotic stresses such as drought, and hence it is distributed in widely varying environments.
Expression profiling is a common step in our current molecular studies to tell us what the cell is actually doing at a point in a time. Many factors determine whether a gene is on or off, such as the time of the day, if the cell is dividing, its local environment, or signals from other cells among others.
Transcriptional programs are important in the development of multicellular organisms and particularly in conifers with very long live cycles, which internal developmental programs should give a co-ordinate response together with seasonal environmental changes and adaptation to unpredictable environmental stresses.
We present a number of tools used in the study of transcription profiles in maritime pine in three different biological situations: (i) analysis of gene expression in specific cells /tissues of maritime pine to identify specific gene families expressed in particular cells or tissues; (ii) transcriptome analysis during an annual cycle of growth to study environmental adaptations in adult trees and (iii) transcriptome-wide analysis of two maritime pine provenances with different geographical origins and phenotypes.

Genomic selection is here, it's not going away, and it's good for you

Steve Lee[1], Joan Cottrell[1], Stuart A'Hara[1], John Woolliams[1], Cosmika Goswami[1], Pablo Fuentes-Utrilla[1].

[1] Forest Research, Northern Research, Roslin, EH25 9SY, Midlothian, Scotland; [2] Roslin Institute, Roslin, EH25 9RG, Midlothian, Scotland; [3] Glasgow University, Scotland; [4] Birmingham University, England

In contrast to some crops and animals, selection and breeding of trees is a relatively recent pursuit. Many tree breeding programmes are only in their first generation with the most advanced in their second or third cycle of selection and breeding. The advantage of this are that tree genetic variation is wide, the scope for making rapid gains through selection of the best genotypes is large, and the experiences and lessons learnt from crop and animal breeders can be immediately applied.

Problems associated specifically with tree breeding include the length of time between testing and release of improved planting stock (generation interval), and the high cost of field trial establishment and maintenance. In an effort to reduce costs and advance gains, tree breeders have introduced indirect selection for end-of-rotation characteristics based on traits expressed at an earlier stage which are well correlated with final crop performance. Good progress has been made in this regard reducing, for example, the selection age for mid-rotation volume to 6-year height in the case of Sitka spruce (*Picea sitchensis* (Bong.) Carr) in Britain. Other cost-saving measures have included studies into the optimum size (number of trees/plot; number of replications) and design (row or block plots; single tree plots) of field-based progeny trials, and the optimum number of replications across sites according to the importance of genotype by environment interaction (GxE). More recently new statistical techniques (e.g. BLUP) have been used to improve accuracy of superior genotypes identification and remove environmental effects.

All these measures have gone a considerable way to reducing the cost-per-year/unit genetic gain of tree breeding. Yet it remains an expensive and lengthy process within an environment of generally decreasing funding. Genomic Selection is a new methodology that is now being applied practically by crop and animal breeders with the potential to drastically reduce costs further and considerably speed up the rate of gain reaching the forest.

Genomic selection (GS), is the very early selection of trees in the laboratory for a suite of characteristics based on DNA-markers known to be well correlated with each of those characteristics at near end-of-rotation. The theory of selection based on properties of DNA has been known for some time, but the cost of searching for the relevant markers has been prohibitive. Over the last decade as new sequencing techniques and ideas have developed, costs have tumbled such that now the GS approach is routinely applied in many commercial dairy-cattle breeding companies. Additional cost-saving measures include the development of SNP DNA-marker panels using RADS (Restriction Associated DNA Sequencing) which negates the need for whole genome sequencing prior to association with phenotypic traits. For the first time, this allows non-model species to be studied.

Work within EU ProCoGen involving tree and animal breeders investigated how the latest GS techniques can be employed with Sitka spruce, a tree species of relatively-minor commercial importance at a European scale. Good correlations have been obtained between RAD generated DNA-markers and 5 or 10-year field grown trees. The results look promising for a future in which the technology is likely to become more reliable and cheaper whilst labour-intensive field-trials become more expensive, and public money decreases. The potential is there for cost-effective and more accurate selection of the genotype, at a very early age, with added gains due to increased selection intensity as large numbers of embryos are screened in the laboratory prior to immediate deployment. Examples will be provided on how this could all be made to work within the British Sitka spruce tree breeding programme.

Tree genome project unexpected outcomes: where science meets society

Nathalie Isabel

Canadian Forest Service (Québec, Canada)

Natural forests dominate the Canadian landscape almost everywhere. They yield most of the wood used by the forest industry and provide numerous ecosystem services. Changing environments, pressure to conserve forest lands, and society's demand for sustainable forest management call for new approaches and practices to increase forest productivity and adaptability.

Around the new millennium, genomic science was seen as a means of developing tools to characterize and help preserve the natural genetic diversity of trees, and more rapidly develop new varieties for reforestation. This vision has become particularly compelling in the context of environmental change and for the adoption of better sustainable forest management practices. Over the last decade, we have witnessed the development of extensive genomic resources (including the know-how) for many tree species. In some cases (genomic selection, climate change adaptation, forest certification, etc.), these newly available data are being used to create tools for translation of results to be used by end-users from across Canada. However, we will face new challenges for which we have to be prepared.

How to start from scratch a genomic characterization (genotyping/sequencing) for a species with little or no sequence resources?

Patricia Faivre-Rampant and Aurélie Bérard

BioMercator: QTL meta-analysis, GWAS representation and candidate gene approaches

Yannick De Oliveira, Lydia Ait Braham, Olivier Sosnowski and Johann Joets

Génétique Quantitative et Évolution – Le Moulon ; INRA – Université Paris-Sud – CNRS – AgroParisTech ; Ferme du Moulon 91190 Gif-sur-Yvette ; France

Compilation of genetic maps combined to QTL meta-analysis has proven to be a powerful approach contributing to the identification of candidate genes underlying quantitative traits. One of the most interesting properties of meta-QTL (or consensus QTL) is its confidence interval (IC) often shorter than IC of corresponding QTLs, decreasing the number of candidate gene to consider. As map compilation and QTL meta-analysis do not rely on genotyping raw data or trait measure, they can be easily achieved even if user holds maps from the literature or genetic databases.
BioMercator was the first software offering a complete set of algorithms and visualization tool covering all steps required to perform QTL meta-analysis. The fourth version of BioMercator propose additional methods and improve graphical representation of large datasets. In this version, user may import sequence and genome annotations datasets within the software in order to display and mine functional annotation related to QTL and meta-QTL.
In order to improve candidates genes detection, we aim to inculde genetic association approach in the new release of BioMercator. Association genetics makes possible to build a relationship between molecular polymorphism and phenotypic variation. Genome-Wide Association Studies (GWAS) present a good potential to extend information provided by QTLs meta-analysis. We integrated GWAS results in Biomercator and provided new functionalities to display and exploit them.

BioMercator V4 is freely available from : http://moulon.inra.fr/biomercator and Biomercator V5 will be available soon.

How to build up a core collection from natural ranges, with the aim of compiling a species reference collection, a discovery panel, or a base for breeding?

Giovanni Vendramin

Can genomics simplify current breeding without the cost of a genome?

Laurent Bouffier[1,2], Marjorie Vidal[1,2,3], Jérôme Bartholomé[1,2], Christophe Boury[1,2], Christophe Plomion[1,2]

[1] INRA, BIOGECO, UMR 1202, 33610 Cestas, France
[2] University Bordeaux, BIOGECO, UMR 1202, 33400 Talence, France
[3] FCBA, Biotechnology and Advanced Silviculture Department, Genetics & Biotechnology team, 33610 Cestas, France

Genomic selection is not a short term perspective for maritime pine. However, molecular markers can be of first importance to enhance innovative breeding strategies. They should be progressively implemented in the maritime pine breeding program. The first step for this implementation is in progress with the genotyping of a part of the clonal archives in order to check identities and pedigrees. Then, forward selections in polycross trials associated with pedigree recovery will be carried out this year to both constitute the breeding population and establish seed orchards. This talk will present the tools developed for identity checking and how we plan to use these molecular markers to enhance innovative breeding strategies.

Why and how to handle genetic diversity in a genomic selection program?

Leopoldo Sanchez

Invited speakers

Bousquet Jean	University of Laval	Canada	jean.bousquet@sbf.ulaval.ca
Bouvet Jean-Marc	CIRAD	France	jean-marc.bouvet@cirad.fr
Cavers Stephen	NERC Centre for Ecology and Hydrology	United Kingdom	scav@ceh.ac.uk
Celedon Jose	University of British Columbia	Canada	jceledon@msl.ubc.ca
Dean Jeffrey	Mississippi State University	USA	jeffdean@bch.msstate.edu
Gonzalez Santiago	INRA	France	santiago.gonzalez-martinez@pierroton.inra.fr
Hickey John	University of Edinburgh - Roslin Institute	United Kingdom	John.Hickey@roslin.ed.ac.uk
Kirst Matias	University of Florida Genetics Institute	USA	mkirst@ufl.edu
Krutovsky Kostya	Forest Genetics and Tree Breeding Büsgen-Institut	Germany	kkrutov@gwdg.de
Mackay John	Oxford University	United Kingdom	john.mackay@plants.ox.ac.uk
Peterson Daniel	Mississippi State University	USA	DPeterson@pss.msstate.edu
Rigault Philippe	Gydle	Canada	prigault@gydle.com
Salse Jérôme	INRA	France	jerome.salse@clermont.inra.fr
Street Nathaniel	University of Umea	Sweden	Nathaniel.Street@umu.se
Ueno Saneyoshi	Forestry and Forest Products Research Institute	Japan	saueno@ffpri.affrc.go.jp
Wegrzyn Jill	University of Connecticut	USA	jill.wegrzyn@uconn.edu
Wilcox Phillip	University of Otago	New Zealand	phillip.wilcox@otago.ac.nz

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

Participants

Abarca Dolores	Spain	mdolores.abarca@uah.es
Arrillaga Isabel	Spain	isabel.arrillaga@uv.es
Arrillaga Isabel	Spain	isabel.arrillaga@uv.es
Avila Concepcion	Spain	cavila@uma.es
Ayoola Emmanuel Akinniyi	Nigeria	tropicalforest2006@yahoo.ca
Bartholomé Jérôme	France	jerome.bartholome@pierroton.inra.fr
Bastien Catherine	France	catherine.bastien@orleans.inra.fr
Bink Marco	Netherlands	marco.bink@wur.nl
Bouffier Laurent	France	bouffier@pierroton.inra.fr
Cabezas Jose Antonio	Spain	cabezas.joseantonio@inia.es
Canlet-Debille Sandrine	France	sandrine.debille@fcba.fr
Cano Maria	Spain	maria.cano@uv.es
Canovas Francisco M.	Spain	canovas@uma.es
Cervera María Teresa	Spain	cervera@inia.es
Chano Victor	Spain	vmchano@gmail.com
de Miguel Marina	France	demiguel@pierroton.inra.fr
Dejardin Annabelle	France	annabelle.dejardin@orleans.inra.fr
Diaz-Sala Carmen	Spain	carmen.diazsala@uah.es
Fabbrini Francesco	Italy	fr.fabbrini@gmail.com
Fossdal Carl Gunnar	Norway	foc@nibio.no
Guerin Vanina	France	vanina.guerin@orleans.inra.fr
Harvengt Luc	France	luc.harvengt@fcba.fr
Heinze Berthold	Austria	berthold.heinze@bfw.gv.at
Ingvarsson Pär	Sweden	par.ingvarsson@umu.se
Joost van Heerwaarden Joost	Nederland	joost.vanheerwaarden@wur.nl
Jorge Véronique	France	veronique.jorge@orleans.inra.fr
Krystufek Vedhu	Austria	vedhu.krystufek@bfw.gv.at
Krystufek Vedhu	Austria	vedhu.krystufek@bfw.gv.at
Li Zhen	Belgium	zhen.li@psb.ugent.be
Lin Yao-Cheng	Belgium	yalin@psb.vib-ugent.be

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

MacKay John	United Kingdom	john.mackay@plants.ox.ac.uk
Mendoza Poudereux Isabel	Spain	isabel.mendoza@uv.es
Merino Sierra Irene	Sweden	irene.merino@slu.se
Miguel Célia Maria	Portugal	cmiguel@itqb.unl.pt
Paiva Jorge Almiro	Portugal	jorge.pinto.paiva@gmail.com
Paulo Joao	Netherlands	joao.paulo@wur.nl
PEGARD Marie	France	marie.pegard@orleans.inra.fr
Pizarro Alberto	Spain	alberto.pizarro@uah.es
plomion christophe	France	plomion@pierroton.inra.fr
Rauda Elza	Latvia	elza.rauda@silava.lv
Rungis Dainis	Latvia	dainis.rungis@silava.lv
Sabatti Maurizio	Italy	sabatti@unitus.it
Sanchez-Rodriguez Leopoldo	France	lsanchezrod@orleans.inra.fr
Santi Frederique	France	santi@orleans.inra.fr
Santos Rodrigues Andreia Sofia	Portugal	sofiasantos@itqb.unl.pt
Savolainen Outi	Finland	outi.savolainen@oulu.fi
Segura Vincent	France	vincent.segura@orleans.inra.fr
Sterck Lieven	Belgium	lieven.sterck@psb.vib-ugent.be
Steve Lee	Scotland	steve.lee@forestry.gsi.gov.uk
Telfer Emily	New Zealand	emily.telfer@scionresearch.com
Teyssier Caroline	France	caroline.teyssier@orleans.inra.fr
Trontin Jean-François	France	jean-francois.trontin@fcba.fr
Tyrmi Jaakko	Finland	jaakko.tyrmi@oulu.fi
Villar Marc	France	marc.villar@orleans.inra.fr
von Arnold Sara	Sweden	sara.von.arnold@slu.se
Woolliams John	United Kingdom	john.woolliams@roslin.ed.ac.uk
Yakovlev Igor	Norway	yai@nibio.no

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

Invited speakers

Bousquet Jean	University of Laval	Canada	jean.bousquet@sbf.ulaval.ca
Bouvet Jean-Marc	CIRAD	France	jean-marc.bouvet@cirad.fr
Cavers Stephen	NERC Centre for Ecology and Hydrology	United Kingdom	scav@ceh.ac.uk
Celedon Jose	University of British Columbia	Canada	jceledon@msl.ubc.ca
Dean Jeffrey	Mississippi State University	USA	jeffdean@bch.msstate.edu
Gonzalez Santiago	INRA	France	santiago.gonzalez-martinez@pierroton.inra.fr
Hickey John	University of Edinburgh - Roslin Institute	United Kingdom	John.Hickey@roslin.ed.ac.uk
Kirst Matias	University of Florida Genetics Institute	USA	mkirst@ufl.edu
Krutovsky Kostya	Forest Genetics and Tree Breeding Büsgen-Institut	Germany	kkrutov@gwdg.de
Mackay John	Oxford University	United Kingdom	john.mackay@plants.ox.ac.uk
Peterson Daniel	Mississippi State University	USA	DPeterson@pss.msstate.edu
Rigault Philippe	Gydle	Canada	prigault@gydle.com
Salse Jérôme	INRA	France	jerome.salse@clermont.inra.fr
Street Nathaniel	University of Umea	Sweden	Nathaniel.Street@umu.se
Ueno Saneyoshi	Forestry and Forest Products Research Institute	Japan	saueno@ffpri.affrc.go.jp
Wegrzyn Jill	University of Connecticut	USA	jill.wegrzyn@uconn.edu
Wilcox Phillip	University of Otago	New Zealand	phillip.wilcox@otago.ac.nz

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

Participants

Abarca Dolores	mdolores.abarca@uah.es	Spain
Arrillaga Isabel	isabel.arrillaga@uv.es	Spain
Arrillaga Isabel	isabel.arrillaga@uv.es	Spain
Avila Concepcion	cavila@uma.es	Spain
Ayoola Emmanuel Akinniyi	tropicalforest2006@yahoo.ca	Nigeria
Bartholomé Jérôme	jerome.bartholome@pierroton.inra.fr	France
Bastien Catherine	catherine.bastien@orleans.inra.fr	France
Bink Marco	marco.bink@wur.nl	Netherlands
Bouffier Laurent	bouffier@pierroton.inra.fr	France
Cabezas Jose Antonio	cabezas.joseantonio@inia.es	Spain
Canlet-Debille Sandrine	sandrine.debille@fcba.fr	France
Cano Maria	maria.cano@uv.es	Spain
Canovas Francisco M.	canovas@uma.es	Spain
Cervera María Teresa	cervera@inia.es	Spain
Chano Victor	vmchano@gmail.com	Spain
de Miguel Marina	demiguel@pierroton.inra.fr	France
Dejardin Annabelle	annabelle.dejardin@orleans.inra.fr	France
Diaz-Sala Carmen	carmen.diazsala@uah.es	Spain
Fabbrini Francesco	fr.fabbrini@gmail.com	Italy
Fossdal Carl Gunnar	foc@nibio.no	Norway
Guerin Vanina	vanina.guerin@orleans.inra.fr	France
Harvengt Luc	luc.harvengt@fcba.fr	France
Heinze Berthold	berthold.heinze@bfw.gv.at	Austria
Ingvarsson Pär	par.ingvarsson@umu.se	Sweden
Joost van Heerwaarden Joost	joost.vanheerwaarden@wur.nl	Nederland
Jorge Véronique	veronique.jorge@orelans.inra.fr	France
Krystufek Vedhu	vedhu.krystufek@bfw.gv.at	Austria
Krystufek Vedhu	vedhu.krystufek@bfw.gv.at	Austria
Li Zhen	zhen.li@psb.ugent.be	Belgium
Lin Yao-Cheng	yalin@psb.vib-ugent.be	Belgium

ProCoGen is financially supported by the European Commission under the 7th Framework Programme

MacKay John	john.mackay@plants.ox.ac.uk	United Kingdom
Mendoza Poudereux Isabel	isabel.mendoza@uv.es	Spain
Merino Sierra Irene	irene.merino@slu.se	Sweden
Miguel Célia Maria	cmiguel@itqb.unl.pt	Portugal
Paiva Jorge Almiro	jorge.pinto.paiva@gmail.com	Portugal
Paulo Joao	joao.paulo@wur.nl	Netherlands
PEGARD Marie	marie.pegard@orleans.inra.fr	France
Pizarro Alberto	alberto.pizarro@uah.es	Spain
plomion christophe	plomion@pierroton.inra.fr	France
Rauda Elza	elza.rauda@silava.lv	Latvia
Rungis Dainis	dainis.rungis@silava.lv	Latvia
Sabatti Maurizio	sabatti@unitus.it	Italy
Sanchez-Rodriguez Leopoldo	lsanchezrod@orleans.inra.fr	France
Santi Frederique	santi@orleans.inra.fr	France
Santos Rodrigues Andreia Sofia	sofiasantos@itqb.unl.pt	Portugal
Savolainen Outi	outi.savolainen@oulu.fi	Finland
Segura Vincent	vincent.segura@orleans.inra.fr	France
Sterck Lieven	lieven.sterck@psb.vib-ugent.be	Belgium
Steve Lee	steve.lee@forestry.gsi.gov.uk	Scotland
Telfer Emily	emily.telfer@scionresearch.com	New Zealand
Teyssier Caroline	caroline.teyssier@orleans.inra.fr	France
Trontin Jean-François	jean-francois.trontin@fcba.fr	France
Tyrmi Jaakko	jaakko.tyrmi@oulu.fi	Finland
Villar Marc	marc.villar@orleans.inra.fr	France
von Arnold Sara	sara.von.arnold@slu.se	Sweden
Woolliams John	john.woolliams@roslin.ed.ac.uk	United Kingdom
Yakovlev Igor	yai@nibio.no	Norway

ProCoGen is financially supported by the European Commission under the 7th Framework Programme