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Nordic Forest Research
(SNS)

Meeting in Iceland August 24-25, 2015

Integrated AdapCAR and EVOLTREE Conference and summer school on ‘Global change and the evolutionary potential of forest trees’

Program and abstracts of presentations

Global change and the evolutionary potential of forest trees

24 August 2015	08:00	09:00	Registration
	09:00	09:15	Opening of the conference Representatives of AdapCAR network
			Adaptation potential of tree species
	09:15	10:00	**Evolutionary trajectories of trees in response to environmental change** *Antoine Kremer, INRA France, Keynote speaker*
	10:00	10:30	**Mapping Ash Dieback disease susceptibility using Associative Transcriptomics** *Andrea Harper, Department of Biology, University of York, United Kingdom. Keynote speaker*
	10:30	11:00	**Coffe break**
	11:00	11:30	**Choosing the right deployment areas for forest regeneration material may contribute to sustain the potential for evolution of forest trees species in a changing environment** *Egbert Beuker, Natural Resources Institute Finland*
	11:30	12:00	**Fine scale adaptation potential in phenology of perennial species with contrasting life history traits** *Albin Lobo, Jon Kehlet Hansen and Erik D. Kjær, University of Copenhagen, Denmark*
	12:00	13:00	Lunch break
	13:00	13:30	**Gene flow in Fraxinus excelsior and implications for conservation** *Devrim Semizer Cuming, University of Göttingen and University of Copenhagen*
	13:30	14:00	**Forest genetic monitoring: an overview of the concepts and definitions** *Barbara Fussi, Bayerisches Amt für forstliche Saat- und Pflanzenzucht, Germany*
	14:00	14:30	**Challenges to evolution-oriented forest management in Scotland** *Richard Whittet, Centre For Ecology and Hydrology, Edinburgh*
	14:30	15:00	**Coffe break**
	15:00	15:30	**Adaptive divergence of drought and cold tolerance related traits in 16 marginal silver fir populations from the French Mediterranean Alps** *Katalin Csillery (ETH, Zurich), Anna Roschanski (University of Marburg), Bruno Fady (INRA Avignon)*
	15:30	16:00	**Genetic analysis of European beech populations across a precipitation gradient using microsatellite markers and SNPs in candidate genes** *Cuervo, Laura; Arend, Matthias; Müller, Markus; Finkeldey, Reiner; Krutovsky, Konstantin*
	16:00	16:30	**"Red" Norway spruce decline – what does the year rings tell?** *Weiwei Huang and Jon Kehlet Hansen, University of Copenhagen, Denmark*

Global change and the evolutionary potential of forest trees

25 August 2015	08:30	09:00	**Hybrid zone of Populus trichocarpa and P. balsamifera in Britsh Columiba and the Yukon in Canada** *Saemundur Sveinsson, Agricultural University of Iceland*
	09:00	09:30	**Hybridization in Icelandic birch: what, when, where and how?** *Kesara Anamthawat-Jónsson, Kesara Anamthawat-Jónsson, Plant Genetics Research Group, University of Iceland*
	09:30	09:45	**Measuring adaptive potential in British populations of Silver Birch, Ash and Rowan** Cristina Rosique, University of Edinburgh
	09:45	10:15	Coffee break
			Genetics and genomics of epigenetics
	10:15	11:00	**Epigenetic mechanisms of plant responses to environment and adaptation** *Igor Yakovlev, NIBIO, Norwegian Institute of Bioeconomy Research, Norway. Keynote speaker*
	11:00	11:30	**Stress-related transcriptional changes and regulation in the Scots pine (*Pinus sylvestris* L.) genome** *Dainis Rungis*
	11:30	12:00	**Telomeres and their connection to tree ageing and regeneration ability** *Tuija Aronen, Natural Resources Institute Finland*
	12:00	12:15	**Gene copy number determination of Pinus sylvestris thaumatin-like protein gene with two different methods** *Vilnis Skipars*
	12:15	13:00	**Lunch**
			Assisted migration
	13:00	13:45	**Time to get moving: Assisted migration in forest trees** *Sally N. Aitken, Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia, Canada. Keynote speaker*
	13:45	14:15	**The Hyrcanian forests in Iran: a gene pool for adaptation of European forests?** *Ole K Hansen, Jørgen Bo Larsen, Palle Madsen, Section for Forest, Nature and Biomass, Faculty of Science, University of Copenhagen, Denmark*
	14:15	15:15	**Coffee break**
	15:15	15:45	**No simple solution: Bioclimatic predictors for success vary between *Quercus*, *Abies* and *Rhododendron* in the Hørsholm Arboretum** *Corrie Lynne Madsen, Erik Dahl Kjær, Anders Ræbild*
	15:45	15:50	**Introduction to the Trees4Future project** *Egbert Beuker, Natural Resources Institute Finland*
	15:50	16:15	**Discussion on the future of AdapCAR - cooperation with entomologists and pathologists**
	16:15		**Poster session**

Evolutionary trajectories of trees in response to environmental change

Antoine Kremer

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There is a large body of evidence stemming from traditional approaches in forest genetics showing that trees have responded to environmental change that occurred during the Holocene, or during most recent times since the little ice age. Taking oaks as an example, this review aims at reconstructing their historical trajectories in order to decipher the evolutionary processes triggering adaptation and the pace at which they were acting. Fossil remains indicate that species extinctions occurred during the very first glacial-interglacial sequences at the early Pleistocene, while remaining species demonstrated efficient microevolutionary responses to cope with recurrent climatic cycles. Assembling data from paleobotany, ecology, genetics and evolution I will show how rapid migration, extensive gene flow, hybridization and local adaptation were the main processes that permitted oak to track climatic warming, since the last glacial maximum. There is concordant evidence between different sources of data that extant differentiation of adaptive traits was mainly generated during the late holocene, and that preexisting historical divergence due to population separation into refugia has been erased by extensive gene flow occurring during colonization. I will further take examples from recent species transfers showing that evolutionary change may occurr at rather short time spans. Finally I will explore how lessons from the historical trajectories may help to make predictions of future responses.

Mapping Ash Dieback disease susceptibility using Associative Transcriptomics

Andrea Harper

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Associative Transcriptomics combines rapid marker discovery for both gene sequence and gene expression variation, with association genetic and systems biology approaches to link markers and genes to traits of interest. The method does not rely on pre-existing genomic resources, which made it particularly suitable for mapping reduced susceptibility to ash dieback in *Fraxinus excelsior*. By aligning mRNA sequence reads from a Danish panel of trees with varying levels of disease susceptibility to a basic gene reference, we were able to identify and score 174,470 novel SNPs, and measure transcript abundance in 32,441 Gene Expression Markers (GEMs). These markers were then analysed to identify significant associations with disease susceptibility scores. The predictive capability of some of these markers was then verified in an independent panel of trees. These and subsequent co-expression analyses also revealed some clues to the genes and pathways that may underlie the reduced susceptibility of some ash trees to this devastating fungal disease.

Choosing the right deployment areas for forest regeneration material may contribute to sustain the potential for evolution of forest trees species in a changing environment

Egbert Beuker.

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Due to climate change the presently used deployment areas for forest regeneration material may not be optimal anymore in the near future. For the temperate and boreal areas in Europe climate warming may result in a shift of the deployment areas mainly in south to north direction or from lower to higher elevation in alpine regions. The human assisted movement of species in response to climate change is called assisted migration. When taking climate change into account when defining deployment areas for regeneration material of commercial forest tree species one is mainly dealing with assisted population migration, the movement of populations (genotypes) within a species-established range.

Assisted population migration has been studied for many decades in provenance trials. They have shown for many tree species a great adaptability to a wide range of climates. This adaptation can be due to changes in the genetic composition of the population (selection), or to phenotypic plasticity. Phenotypic plasticity is defined as the range of phenotypes a single genotype can express as a function of its environment. It has proven difficult to conclusively distinguish whether phenotypic changes are genetically based or the result of phenotypic plasticity, and thus the relative contribution of genetic change and plasticity are unknown. Because plastic changes can occur within a generation and evolutionary changes necessarily occur across generations, one prediction is that plasticity may be a more important immediate response to very rapid environmental changes. This is especially valid for long living plants, such as trees. Therefor it would be of value to be able to select for plasticity. Unfortunately still little is known about the genetic background of plasticity.

For the new deployment areas for Scots pine in Finland, the transfer models used were developed by SkogForsk in Sweden and are based on extensive data sets from provenance experiments in both Finland and Sweden.

Fine scale adaptation potential in phenology of perennial species with contrasting life history traits

Albin Lobo, Erik Dahl Kjær, Lars Nørgaard Hansen and Jon Kehlet Hansen

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Keywords: Climate change, phenology, genetic variation, local adaptation

We assessed and compared the genetic variation within and among populations in phenology, survival and growth of woody perennials based on observations in common garden trials. We studied 12 species with contrasting life history traits and compared differences among species in the patterns. The trials include progeny from a number of single tree collections from putative native populations sampled within Denmark. The 12 species representing different botanical genera, distribution patterns and life history traits are; *Acer campestre, Betula pubescens, Cornus sanguinea, Frangula alnus, Fraxinus excelsior, Malus sylvestris, Prunus avium, Prunus spinosa*, *Quercus petraea, Rosa dumalis, Tilia cordata* and *Viburnum opulus*. Genetic parameters in growth, time of bud burst scores and leaf senescence scores were estimated based on co-variance within and among trees in open-pollinated family groups. Degree of population differentiation was estimated in both absolute values and as *Qst* values. We estimated the predicted response from a selection of 1% and 5% and compared these values with the present variation between populations. For each species, we tested if spring and autumn phenology of populations correlated with minimum temperatures in spring and autumn respectively at their site of origin. We also estimated correlation between phenology and growth. The species showed different patterns. Narrow sense heritability and level of genetic differentiation varied substantially among species. The level of heritability and additive genetic variance suggested presence of high adaptive potential in most (but not all) of the investigated species for the phenological traits if exposed to strong selection. However, while *Quercus petraea* revealed significant positive genetic correlation between growth and bud burst, this was not the case for any of the other species in the present study. The implications in relation to current considerations in forest management on assisted migration and genetic enrichment as a response to global warming are discussed.

Gene flow in *Fraxinus excelsior* and implications for conservation

Devrim Semizer Cuming[1, 2], Erik Dahl Kjær[2] and Reiner Finkeldey[1]

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Gene flow is an important determinant for the genetic structures of populations. While high gene flow works on homogenizing genetic structures, low gene flow allows non-random distribution of alleles and genotypes. In wind-dispersed species, such as Fraxinus excelsior, pollen and seed can move further in fragmented landscapes than in continuous forests which may affect the adaptation potential of the tree species. Cultivation of ash plantations close to interfertile native trees may also have an impact on surrounding natural populations by potentially reducing genetic variation and adaptability of later generations. On the other hand, it may allow the spread of adaptive traits among and within populations, such as in the case of ash dieback caused by Hymenoschypus fraxineus in Europe. The pathogen is currently threatening the species in various ages on a continental scale epidemically, raising an ecological and economical concern about the species' disappearance from the forest ecosystems. Even though some ash trees show resistance to the pathogen, the spread of resistance at a population genetic level cannot match the speed of pathogen spread, simply because ash is a long living species and typically takes 10 years to flower. Therefore, the speed of population recovery will largely depend on the extent of pollen- and seed-mediated gene flow.

To guide genetic management of ash, including its conservation and reproductive material use, we studied gene flow dynamics in Rösenbeck, North Rhine-Westphalia, Germany. The molecular genetics analyses were carried out using 12 nuclear microsatellites. Parentage analyses mainly indicated the short distance (up to 100 m) pollen and seed dispersals, localized in the natural forest itself, a very limited gene flow from the planted trees, and 25% gene flow from the landscape. These findings show importance for practical forestry before the establishment of gene conservation stands, and enrichment plantings around unhealthy stands.

FORESTS GENETIC MONITORING: AN OVERVIEW OF CONCEPTS AND DEFINITIONS

Barbara Fussi[*1], Marjana Westergren[2], Filippos Aravanopoulos [3], Roland Baier[1], Darius Kavaliauskas[1], Domen Finžgar[2], Evangelia Avramidou[3], Monika Konnert[1], Hojka Kraigher[2]

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Key words: indicators, verifiers, genetic diversity, management, forest genetic resources, FGM

Sustainable forest management is based on the long-term adaptability of forest ecosystems to the changing environment, and starts at the gene level. Therefore the goal of the convention on biological diversity, to halt the ongoing erosion of biological variation, is of utmost importance for forest ecosystem functioning. Monitoring biological diversity in general over time is needed to detect changes that can threaten biological resources. Genetic variation is the basis of biological diversity and needs special attention, also in the form of monitoring. Forest genetic monitoring (FGM) gives a possibility to detect potentially harmful changes of forest adaptability before they are seen on higher levels and it can improve or verify sustainable forest management. Theoretical concepts of genetic monitoring have been developed and need evaluation for implementation at an international scale. Here we give an overview of the concepts and definitions of FGM, schematically describe the optimization and implementation of a forest genetic monitoring concept and propose an improved definition of FGM.

Challenges to evolution-oriented forest management in Scotland

Richard Whittet

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In recent years, British seed sourcing practice has tended to focus on the principal that “local-is-best” for native woodlands and on material from breeding programmes for commercial forestry. However, in the face of continuing and accelerating global change, the suitability of these strategies has been called into question.

In this talk, I will discuss possible alternative policies for choosing seed sources and planting stock for new planting in Great Britain and analyse whether Britain is ready to implement revised guidelines based on our understanding of environmental change, knowledge of variation within tree species and the current configuration of our plant and seed supply sector. I will conclude by indicating changes that would be necessary to integrate continuing adaptation to environmental change into forestry practice.

Adaptive divergence at drought and cold tolerance related traits in 16 marginal silver fir populations from the French Mediterranean Alps

Katalin Csillery[1], Anna Roschanski[2], and Bruno Fady[3]

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We estimated adaptive divergence among 16 marginal silver fir populations from the French Mediterranean Alps using a Qst-Fst approach. Phenology, growth and drought and cold tolerance related phenotypic traits were measured in a common garden experiment conducted between 1997 and 1999 at a low land location near Aix-en-Provence, France. A randomized complete block design was used, where families from different provenances were either treated as a control or exposed to drought stress. Genetic data was available for ten isozyme loci and, additionally, for 267 SNP loci from 175 candidate genes in four populations. Our results highlight the importance of some marginal populations for conservation genetic/assisted migration purposes.

Genetic analysis of European beech populations across a precipitation gradient using microsatellite markers and SNPs in candidate genes

Laura Cuervo[1], Matthias Arend[2], Markus Müller[1], Reiner Finkeldey[1], Konstantin Krutovsky[1]

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Climate change scenarios for Europe predict an increment in the annual mean temperature and a decrease in precipitation during summer months, increasing the risk of droughts. Since European beech (*Fagus sylvatica*) is one of the most important species in Europe, the adaptive potential of this species to climate change is of great interest. With the aim of assessing the genetic variability underlying drought stress tolerance of *F. sylvatica,* both saplings and adults from beech populations located along two precipitation gradients in Switzerland were sampled. Individuals were genotyped at ten random nuclear microsatellites, three EST-linked microsatellites and 70 SNPs in 23 candidate genes associated with drought related traits. Analyses of microsatellite markers demonstrated that the investigated populations have high genetic diversity. No significant differences regarding allelic richness and heterozygosity were found. There is low but significant population differentiation among the populations, and populations occurring in sites with low precipitation had the highest pairwise differentiation. The lowest genetic differentiation was found at EST-linked microsatellites and SNPs markers, which may indicate that the populations are under balancing selection. F_{st} outlier analysis identified two SNPs as candidates for balancing selection and five SNPs as candidates for positive selection. This study willcontribute to a better understand of the genetic basis of drought stress tolerance of *F. sylvatica*.

“Red” Norway spruce decline – what does the year rings tell?

Weiwei Huang and Jon Kehlet Hansen

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Keywords: genetic variation, climate change, adaptation

In the late 1980ies and early 1990ies many trees in plantations with Norway spruce (*Picea abies*) showed a severe decline where current-year needles first became bright and later red. A genetic variation was found between landraces and newly imported provenances and between individual trees. A single abiotic factor triggering the decline was never found and biotic factors were ruled out.

The objective of this study was to test if trees showing different degrees of decline in 1989 showed different responses in growth to variations in climate and to extreme climatic events. Wood cores were sampled from plus trees of Norway spruce in seven Danish plantations in 1986/1987 for ring width- and wood density measurements. At this time there was no sign of decline in the plantations. The plus trees were revisited in 1989 where “red” Norway spruce decline symptoms were present.

Growth was positively correlated with the drought index in June and July and a negative correlation was found between growth and mean temperature in September last year at one site, and with a tendency to a negative correlation at five other sites. There were no indications of different responses to climate variations between trees with different decline scores. However, the number of trees with severe decline in the study is small, and other studies have shown a tendency to a larger decrease in growth after drought years among Norway spruce trees that later showed the “red” Norway spruce decline. Plans for further studies are discussed in this context.

Genome permeability between two poplars: the large scale hybrid zone between Populus balsamifera and P. trichocarpa in British Columbia

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The hybrid zone between black cottonwood (*Populus trichocarpa* Torr. & A.Gray ex Hook.) and balsam poplar (*P. balsamifera* L.) in northern British Columbia (BC), Canada and Alaska is well known but poorly characterized. Here we use genotyping-by-sequencing (GBS) to examine the geographical structure of this hybrid zone. We examined 23 populations of poplar trees in a latitudinal transect from the Yukon (62°N) to southern BC (49°N), sequencing reduced representation libraries from six - eight individuals per population to provide a survey of single feature polymorphisms (SFPs) across the genome. The variation takes the form of a stepped cline with a transect contact zone near the Peace River in northern BC. However evidence of hybridity extends for hundreds of kilometers north and south of the contact zone indicating both a partial barrier to hybridization and extensive genome permeability. The morphological characteristic of carpel number correlates well with genetically determined hybrid index and is highly useful taxonomically. Extensive allelic transfer has occurred between these species through the hybrid zone, making a taxonomically confusing situation. In fact, most trees in northern and interior BC are likely to be genetically admixed to some degree.

Hybridization in Icelandic birch: what, when, where and how?

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Introgressive hybridization between European downy birch (*Betula pubescens* Ehrh.) and dwarf arctic birch (*B. nana* L.) has been confirmed in Iceland but limited knowledge on the extent or timing of such hybridization exists. In this presentation I intend to give an overview of our work from the first chromosome identification of natural triploid hybrids between these two co-existing species, to the detailed morphological and molecular analyses of hybrid introgression in woodlands in Iceland with reference to populations in Scandinavia, Scotland and Greenland. I will also talk about our studies on Holocene birch hybridization using subfossil pollen from lake sediments. These studies also show that the Holocene birch hybridization in Iceland coincided with periods of climate warming, meaning that with the ongoing and ever-increasing global climate change we can expect a new phase of woodland expansion and hybridization.

Measuring adaptive potential in British populations of Silver Birch, Ash and Rowan

Cristina Rosique

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Rapid climate change is a significant threat to the long term persistence of native tree populations.

Concern has been expressed that tree populations might fail to adapt due to rate of change compared to tree longevity, insufficient adaptive variation in tree populations and the impermeability of the landscape to dispersal. In contrast, others have contended that most tree species have high phenotypic plasticity maintain high levels of within-population genetic variation and exhibit effective gene dispersal capability, all characteristics which should enable an adaptive response.

To assess the potential for adaptive mitigation in forest tree populations we need to focus on quantifying patterns of adaptive genetic variation and the likely response of such variation to environmental change.

To understand possible responses of British trees to climate change, we must address a significant knowledge gap regarding patterns of adaptive variation in native tree species. In this talk, I will introduce my PhD research project which involves assessments of adaptive trait variation within experimental provenance trials of silver birch (*Betula pendula),* ash *(*Fraxinus *excelsior*) and rowan (*Sorbus aucuparia*) across Great Britain.

Epigenetic mechanisms of plant responses to environment and adaptation

Igor Yakovlev

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Plants, as sessile organisms, are constantly challenged by environmental perturbations. Recent studies have provided multiple evidences indicating that epigenetic mechanisms are indeed essential for stress memories and adaptation in plants.

Epigenetics is defined as heritable changes in gene activity and expression that occur without alteration in DNA sequence and refers to the “orchestration” of genomic regulation by dynamic and coordinated modifications. These non-genetic alternations are mostly regulated by two major groups of epigenetic modifications: chemical modifications of DNA and histone proteins modifications. Epigenetic modifications, resulting from a dynamic process controlled by multiple mechanisms responsive to environmental stimuli can remain stable and heritable. Epigenetic mechanisms include multiple histone modifications (including, acetylation, methylation, phosphorylation, ubiquitination and sumoylation, etc.), DNA methylation, small and non-coding RNAs, and chromatin structure changes. These mechanisms, in addition to other transcriptional regulationary events, ultimately regulate gene activity and expression during development and differentiation, or in response to environmental stimuli. According to current models, chromatin remodelers, histone modifiers and DNA methylating/ demethylating activities interact and influence each other’s performance, and their interactions are often mediated by both short and long non-coding RNAs (NcRNAs).

Understanding how epigenetic regulation is involved in plant response to environmental cues is highly important, not just for a better understanding of molecular mechanisms of plant stress response but also could open stunning new capabilities in agriculture, medicine, and all practical applications of biology.

We mostly focus in our talk on molecular mechanisms of epigenetic memory in Norway spruce and will discuss the growing list of environmentally-mediated epigenetic modifications and enzymes involved, and examples of transgenerational epigenetic inheritance events that may begin to change our views of adaptive responses to the environment and evolution.

Stress-related transcriptional changes and regulation in the Scots pine (*Pinus sylvestris* L.) genome

Rungis D., Voronova A., Krivmane B, Skipars V., Rauda E., Snepste I., Veinberga I.

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Scots pine is a long-lived organism, with a long generation time, which requires the tree to adapt to a wide range of growing and environmental conditions. Conifer genomes are characterised by multiple gene families and pseudogenes, contain large inter-gene regions and a high proportion of repetitive sequences.

Conifer genomes contain a high proportion of retrotransposable elements (REs), and genes with large introns (up to 100 kb), that contain various types of mobile genetic elements. Most RE families in conifer genomes are old and inactive, however, some highly represented REs have conserved sequences and may be still active. Transcription and transposition of REs is associated with stress conditions in various plant species. However, expression of the RE does not directly imply further transposition. In conifer genomes, it is possible to detect RE sequences co-expressed with stress associated genes as part of their introns. Alternatively, RE sequences could function as long non-coding RNAs, initiate transcription with their cis-acting elements, or are expressed with other untranslated regions of the genome. Our studies have shown that RE-like sequences are differentially expressed in various stress conditions.

MicroRNAs (miRNAs) are ~20-24 bp non- protein coding RNAs that play an important role in many biological and metabolic processes, including regulation of gene expression. There are only a few reports of miRNA studies in conifers, and there is no published data about Scots pine microRNAs. Using the IonTorrent PGM, we identified potential mature miRNAs in Scots pine, which need to be further characterised and validated.

Another source of genetic variation is gene copy number variation (CNV), which has been linked to variations in gene expression levels, and CNV is a major component of genetic variation within the human genome. CNV of stress-related genes in the Scots pine genome was investigated using a variety of approaches, as well as correlation with stress-induced gene expression levels.

These role of these various genomic mechanisms in genomic plasticity and adaptation to stress conditions in Scots pine needs to be further investigated.

Telomeres and their connection to tree ageing and regeneration ability

Tuija Aronen

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Eukaryotic chromosomes are formed of a single DNA molecule, which terminates in specialized heterochromatin called telomeres. The function of telomeres is to protect chromosomes from degradation and fusion during DNA replication. Shortening of telomeres has been connected with ageing and loss of cell replication or regeneration capacity. The information on the role of telomeres in ageing of plants is limited, and telomeres of long-living trees have only been studied in a few species. In addition, biotechnological methods are applied more and more also in tree species. Transgenic trees are studied, while clonal propagation by tissue culture is already a standard practice in many species, and clones can be maintained under tissue culture conditions or cryopreserved for long times. There is, however, no data existing on the effects of tissue culture and its duration, or stress factors involved in biotechnologies, on telomere length in long-living trees.

Firstly, the aim of this study was to determine the variation in telomeric repeats during the life-span of Scots pine (*Pinus sylvestris* L.) by studying different tissues and age groups ranging from immature embryos to cambium, buds, and needles of mature, 200-year-old trees. The telomeric repeats in Scots pine including interstitial and centromeric repeats ranged from 0.9 up to 25 kb, and true telomeres were evaluated to have a mean length of 19.3 kb (±SE 0.17). Telomeres were observed to shorten with increasing tissue differentiation, embryonal samples having the longest repeats with an average length of 21.1 (±0.34) to 21.7 kb (±0.42) and the needles having, on average, the shortest repeats of 18.1 kb (±0.24). After germination, ageing per se had no significant effect on the length of telomeric repeats in cambium, bud, or needle tissues. In the older trees (50–200 years of age), the telomeres in stem cambium showed shortening towards the tree top. This is the first observation on such a positionrelated variation in telomeric repeats. Finally, there was a remarkable genotypic variation in the length of telomeric repeats, and this was consistent over the tissue types.

Secondly, potential effect of tissue culture and cryopreservation on tree telomeres was studied studied in silver birch (*Betula pendula*) using clonal materials consisting of different-aged outdoor trees and tissue cultures of seven genotypes. In addition, the effect of cryopreservation and long-term culture was examined by comparing 12 embryogenic cultures of Norway spruce (*Picea abies*) prior to and following cryostorage. In silver birch, no correlation of ageing and the length of telomeric repeats was found when germinated seeds, and leaf and cambium samples from 15- and 80-year-old trees were compared. Positional variation in the telomere length was, however, observed in the cambium of mature trees, the stem base having longer repeats than the upper parts of the tree. Tissue cultures were found to have shorter telomeres than outdoor trees: prolonged culture, callus culture and stressful conditions were all observed to shorten telomeric repeats and should thus be avoided in birch micropropagation. There were significant differences among the studied silver birch genotypes in their telomere length, and these differences were consistent over the sample types. In Norway spruce, successful cryopreservation was not found to affect the length of telomeres in the embryogenic cultures. In non-regenerating cultures, however, telomeric DNA was observed to be severely damaged. Significant genotypic differences among the SE lines in their telomere length were found also in Norway spruce. More detailed studies in Norway spruce are going on.

References:

Aronen & Ryynänen: Variation in telomeric repeats of Scots pine (*Pinus sylvestris* L.). Tree Genetics & Genomes (2012) 8:267–275

Aronen & Ryynänen: Silver birch telomeres shorten in tissue culture. Tree Genetics & Genomes (2014) 10:67–74

Determination of copy number variation of the *Pinus sylvestris* thaumatin-like protein gene using multiple detection methods

Vilnis Šķipars

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Copy number variations (CNVs) have been a hotspot in research on the impact of polymorphisms in the human genome on pathology. In forest tree species, CNVs have been studied much less. Emerging molecular genetics technologies like next generation sequencing and digital polymerase chain reaction (dPCR) are becoming more available to forest genetics researchers, increasing the capacity of this scientific community to analyse CNVs of forest tree species despite of the complexity and size of their genomes. Existence of current conifer breeding programs in Latvia prompt us to use these advances to gain otherwise unavailable insights into the molecular genetic characteristics of individual trees involved in the breeding programs for potential use in further studies and, eventually, practical application of gained information as a selection criterion. However, due to lack of well characterised reference material, the task of gene copy number variation analysis in conifer genomes is complicated.

Quantitative polymerase chain reaction (qPCR) is regarded as the golden standard for assessment of CNVs and is often used to verify the results of other methods. Some data about differences in gene copy numbers of *Pinus sylvestris* thaumatin-like protein gene (*PsTLP*) between Scots pine individuals have been obtained previously in our laboratory using qPCR. But, as no reference material existed when the analyses were performed, it was necessary to validate the results. dPCR is a new, promising tool with increased discriminatory power compared to qPCR. This method provides the results in absolute copies of a target sequence in a volume of sample thus allowing calculation of the absolute gene copy number if the genome size of the species is known. In addition, the comparative high resolution melting curve analysis (C-HRM) method was also used for CNV detection. C-HRM is a method which indicates the deletion or amplification of a target gene by comparison of the peak height of the target sequence to the peak height of a reference sequence in a melting profile and allows simultaneous detection of single nucleotide polymorphisms (SNPs) and other mutations.

Four samples previously studied by qPCR method were later analysed with dPCR and C-HRM. dPCR results strongly correlated with the results obtained with qPCR (R=0.92). C-HRM results also correlated with the previously gained results for the same four samples (R=0.99). Yet when a larger number of samples (n=28) is compared (qPCR results *versus* C-HRM results) the R value drops to 0.65. We believe this effect is due to lack of a reference sample in determination of gene copy number by qPCR. Selection of five samples with lowest qPCR results and three samples with highest qPCR result shows a very strong correlation with C-HRM data (R=0.93). We are going to perform additional dPCR analyses to better characterise the correlation between qPCR and dPCR results.

The obtained data allow us to conclude that existence of gene copy number variations for Scots pine thaumatin-like protein gene is extremely likely. Combination of qPCR and C-HRM or dPCR for analysis of a set of samples results in a number of samples indicated as having increased *PsTLP* gene copy number by more than one method.

Time to get moving: Assisted migration in forest trees

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Climate change is disrupting the geographic match between tree species and populations, and local environments. The long-recognized practice of collecting seed locally for reforestation and restoration is becoming inadequate for ensuring forest health and productivity. Species ranges are expected to shift, with rear edge populations collapsing due to both biotic and abiotic factors, and new geographic areas becoming suitable for range expansions faster than species can migrate. Assisted species migrations are being considered by forest managers in some geographic areas, and implemented in other jurisdictions, to assist the colonization of new areas. The effects of such species migrations involve primarily ecological rather than genetic factors and decisions.

For many wide-ranging species, large areas are forecast to remain within the species climatic niche, but local populations are predicted to no longer be well adapted. In such areas, reforestation or restoration can continue with the same species, but when seedlings are planted, seed should be collected from source populations adapted to new conditions. This practice, termed assisted gene flow (Aitken and Whitlock 2013), can be used to facilitate the adaptation of tree populations to new climates. Temperate and boreal forest trees are ideal candidates for assisted gene flow as 1) populations of most species are locally adapted to climate; 2) there is a long history of characterizing local adaptation in provenance trials: and 3) species show similar patterns of adaptation along climatic gradients (Aitken and Bemmels 2016). Potential negative effects of assisted gene flow include disruption of local adaptation to non-climatic factors and outbreeding depression, but for temperate and boreal tree species these risks are low relative to the extent of maladaptation from climate change.

Basic strategies for assisted gene flow can be developed using information from field-based provenance trials; seedling common gardens; genome scans for genotype-environment associations; generalized patterns of local adaptation in sympatric species; or climate models. Composite provenancing, the mixing of seed from different provenances, can also be used to buffer uncertainty around future climates. Assisted gene flow strategies can be refined over time using new species-specific information as it becomes available, but this practice should be implemented now whenever seedlings are planted for reforestation or restoration.

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The Caspian forests of Iran: A gene pool for the adaptation of European forests?

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The Caspian forests

South of the Caspian Sea and north of Tehran in Iran is the east-west mountain range Elburz. In these mountains the Caspian forests cover a total area of approximately 1.8 million ha, of which 100-200,000 ha is estimated to be virgin forest. The forests contain many of the same tree and shrub species that we have naturally in Denmark and Europe. It is species' like beech, ash, oak, maple, lime, wild cherry, elm and hornbeam.

The forests date back more than 2.6 million years; i.e. back to the time before the ice ages began their regular advances and retreats of the ice masses farther north in Europe. The Caspian forests has thus existed continuously during all ice ages and interglacial periods, and consequently the forests' and their genetic diversity have only to a lesser extent been affected by the climate fluctuations of the ice ages compared to corresponding deciduous forests in Europe, North America and the Far East. Particularly in Europe ice ages exterminated species or reduced population sizes so much that the genetic variability has been reduced significantly.

As a result of the above, the Caspian forests is probably the largest forest area of temperate deciduous forest containing European flora which has not, or only to a lesser extent, been exposed to the two factors that empirically reduce both the number of species as well as gene pools the most - ice ages and human exploitation.

A new research initiative

We are seeking to establish a new research initiative with the overall specific goal of testing whether the Caspian forests contain an important genetic potential for our European forests and their ability to adapt to future challenges in terms of climate change and new diseases and pests. A more general aim of the project is to contribute to the understanding of forest evolutionary processes as a function of selection pressure (ice ages/human influence) and isolation (refugia conditions).

In the first place, we are applying for funding to establishment of provenance trials on afforestation areas in Denmark and Iran. From Iran is collected from about 6 species each with 8-10 provenances each. The trials will also include European/Danish reference provenances of the species found in both regions. There will be made quantitative genetic analyzes of the test material in 'common garden tests'. As support for the designation of stands in Iran as well as for comparison of genetic diversity between the Caspian and European forests DNA analysis methods will be applied.

No simple solution: Bioclimatic predictors for success vary between *Quercus*, *Abies* and *Rhododendron* in the Hørsholm Arboretum.

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Species distribution modeling (SDM) is an important tool to assess the impact of global environmental change and in predicting the current or future distributions of species. This study aims to improve model projections to target assisted migration through investigating whether 1. a common set of bioclimatic predictor parameters can be identified, or 2. a more specific approach using different predictor parameters for different genera should be applied. We analyze the impact of 19 bioclimatic predictor variables and altitude from the WORLDCLIM database on introduction success of *Quercus*, *Abies* and *Rhododendron* using dendrometric measures from the Arboretum in Hørsholm, Denmark. Linear regression analyses revealed no shared significant predictor variables for growth between *Quercus* and *Abies*. Logistic regression analyses of factors affecting survival revealed isothermality as the single common predictor variable between all genera. The Principal Component Analysis (PCA) revealed a large cluster of negatively correlated predictor variables for *Quercus*. The most important predictors for growth in *Abies* were Isothermality and Mean Temperature of Wettest Quarter with the most successful trees from Europe and U.S.A., and least successful collected from Japan and South Korea. Both linear and logistic regression showed a clear difference in significant predictor variables between genera, indicating that SDM predictions may be more accurate with the application of a "customized" set of predictor parameters.