evoltree

EUROPEAN PROJECT ON FOREST BIODIVERSITY

News 2008 | Number 2

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evoltree EXTERNAL NEWSLETTER

evoltree is an interdisciplinary network of scientists in Europe. This Network involves 25 research groups from 15 European countries that are working together to identify and study genes of adaptive significance in order to evaluate the contribution they make to the evolution of tree species and tree communities. Research will also be carried out on organisms that interact with trees such as insects and mycorrhizal fungi.

www.evoltree.eu

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A. INTRODUCTION

Message of the coordinator

by Antoine Kremer, evoltree coordinator

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EVOLTREE is at the crossroads. After two years of intensive efforts to get the various existing resources organized and structured, the network has now come to the stage of a scientific and technical platform for Ecosystem Genomics. Tools developed within the integration activities are becoming operational to provide services and information to the different partners. A significant number of databases were constructed and aggregated on the portal and the eLab of evoltree. The repository centre is regularly feeded with DNA samples and other genomic libraries. The third year has also witnessed the increase of training activities. Fifteen summer courses and workshops are planned within year 3 of the project.

I was very pleased to hear that the European Commission has announced a topic on "Forest Ecosystem Genomics" in its next call for 2009. The call is for a Coordinating and Support Action and not a Research Project. It aims at strengthening European-North American cooperation in our research field. "The purpose of this topic is to facilitate scientific cooperation between Europe and North America on forest genomics with a view of identifying common research objectives, developing jointly innovative science plans. The adaptation of forests to the expected climate and environmental changes over the next 100-200 years should be targeted". I see this call as a clear support of the Commission to the activities implemented by evoltree and as a positive signal for durable integration of the network. Of course evoltree will make a proposal in response to the call.

Ferns and mosses on forest floor (Photo by Sascha Rösner | fotouristen.de).

B. PROGRESS IN INTEGRATION ACTIVITIES

Development of EST sequences

by Christophe Plomion

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The genomes of trees are vast and complex, with a lot of repeating and non transcribing DNA. It is therefore not feasible at the moment to obtain their complete sequences. Thus, one of evoltree's aims has been to develop several genomic resources that focus only on the parts of the genomes that are helpful to reveal the evolutionary history of different tree species. One approach is to cut the genomes into chunks of workable size, for example, in BAC (Bacterial Artificial Chromosome) libraries or cDNA libraries (libraries that contain only the part of the genome that has actually been transcribed, or complementary DNA - cDNA).

One such BAC library has been developed as a resource for structural genomics research in oak. This library contains 90,000 clones each carrying on average between 120-150 kb oak DNA. As the estimated genome size of oak is 740Mb/C, the library provides approximately 8 haploid genome equivalents of each phase. A sample sequencing of 15,000 BAC ends and 5 allelic BACs will provide a first snapshot of the structure of the oak genome, essential information before a whole genome is being sequenced.

A second important approach to handle the trees' vast genomes is to find small, representative parts of the sequences of interest that in subsequent studies can be obtained instead. For this task several genetic markers have been developed (e.g. microsatellite markers) for several insect and fungi species to survey their genetic diversity, as well as SNPs (single nucleotide polymorphysm) that will be used for association and comparative mapping in three conifer species. This latter set of markers has been developed in collaboration with a NSF funded project led by David Neale, University of California Davis (link[1]).

The main emphasis during the last two years, however,

A BAC library has been developed for oaks containing 90,000 clones (Photo by Sascha Rösner | fotouristen.de).

has been laid on the development of a catalogue of expressed sequence tags (ESTs) in trees. EST databases are an important source of information for the selection of candidate genes – genes that are potentially responsible for the expression of a trait such as drought hardiness. With the help of mapping and association studies, the researchers can eventually find out which of the candidate genes are in fact contributing, and to what extent, to the expression of these traits. So far 200,000 sequence reads have been obtained for *Quercus robur* and *Quercus petraea, Fagus sylvatica, Fraxinus excelsior, Pinus pinaster* and *Pinus sylvestris*. Another 50,000 reads have been obtained for insects (*Lymantria dispar* and *Lymantria monacha*) and 350,000 for mycorrhizal fungi (*Glomus intraradices*, *Laccaria bicolor*, *Lactarius quietus* and *Tuber melanosporum*). To analyse this vast amount of data, a bioinformatic pipeline has been developed that allows i. organization of each species gene index into a unigene set for further molecular investigations, ii. the functional annotation of these collections and iii. the in silico (computer based) mining of simple sequence repeats (SSRs) and SNPs for further wet lab development.

Starting with EST sequences, the methodology for obtaining full gene and promoter information is laborious. In a pilot study we therefore generated gene-enriched genomic libraries for *Picea abies* and *Quercus robur* using hypomethylated partial restriction procedures. During this process, repetitive DNA is eliminated from the genomic libraries while low copy DNA (i.e., genes) is recovered. About 10,000 reads were obtained for each species and are now being scrutinized.

Besides developing new EST's, resequencing of known candidate genes is underway in the Pinaceae (40 genes in 23 conifers + 140 genes in 12 pine species) and oak (1,000 genes) to discover allelic diversity.

To make these vast and growing resources available to all participating scientists, a library management system has been set up. With this system, it is now possible to store, track, and query the different libraries, most of which (and their associated clones) have been made available at the repository center in Vienna (link[2]).

Update on the eLab. How it functions and what type of data is currently accessible

by Dieter Kopecky

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The eLAB prototype, which was discussed in Newsletter 2008/1, has been improved, extended and brought into routine work. This issue provides an update on the currently available datasets and the work being undertaken in the bioinformatics working group.

Very large amounts of heterogeneous information, data and knowledge are continuously being generated by evoltree research activities. In addition, datasets containing similar type of data maintained by partners or external collaborators have been made available for sharing. All these distributed electronic resources are being integrated into a single information system called eLAB, which is available at the project webpage, with access limited to registered users (link[3]). Within this framework, thematic databases are developed by the most experienced partner in this field and populated with research results from all partners. The eLAB provides an Internet search portal allowing integrated searches over all thematic databases. Figure 1 gives an overview of the distributed data source and data qualities being integrated within the eLAB.

Currently, it allows searches against the annotations stored within the EST sequence database developed by CBiB in Bordeaux, as well as the corresponding material information held by the repository centre at ARC. For example, somebody could be interested in finding all chloroplast-related ESTs for *Fagus sylvatica*. Specifying this query within the eLAB, the user will obtain the requested ESTs, their sequences as well as all the information coming from the repository centre, such as gel pictures, quality control information, etc.

The integration of the GeneBank database of INRA Orleans

Figure1. The architecture of the eLAB.

holding data about mapping pedigrees and reference to natural or breeding populations has also been finished. The embedding of phenotypic, genotypic and map data from INRA Pierroton and the University of Southampton is currently ongoing.

In addition to the integrated search, the eLAB currently provides a BLAST search interface against all ESTs that are being sequenced at CNS and are part of the CBiB library database. Therefore, in addition to searches by annotation, ESTs can also be found by sequence similarity.

The sources of the data made available by the eLAB are the local domain databases and information systems, which have already been described in Newsletter 2008/1 and have been further improved by various partners. In order to keep the navigation between the involved information systems easy, a single-sign-on solution has been implemented, which requires only a single login at the eLAB portal and, thereafter, all involved information systems can be accessed transparently without a new login.

The ISS information system has been largely improved. In addition to the input of various metadata sets in several ISS, the underlying detailed datasets are now automatically transferred from the local ISS databases to the central ISS Metadata Base, from where links between the metadata items and the actual datasets are available.

The Genebank Information System has also become publicly available and data are continuously entered into the system. Two installations of cMAP, a comparative genetic and physical map viewer, have been installed at INRA Pierroton and the University of Southampton and will provide genetic maps for various species. QuercusMap, an information system for managing phenotypic and genotypic data about oaks, has been made available at INRA Pierroton and is currently adopted for other species at the University of Southampton.

All the described systems will be soon fully integrated within the eLAB and will enable searches across various types of information in biodiversity.

Update of what is currently stored in the repository

by Sylvia Fluch

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The repository centre for DNA reference material situated at the Austrian Research Centers GmbH (ARC) is continuously expanding the collection of reference samples. Mate-

Elm (*Ulmus glabra)* with seeds, Białowieża National Park, Poland (Photo by Sascha Rösner | fotouristen.de).

rial from different partners and various species is shipped to the central spot for quality assured material storage and redistribution. Currently, 185.973 different samples of ESTs from six different partners are available. These resources were all being sequenced at CNS and DNA extracted at ARC for microarray production. Among evoltree resources, available ESTs and thus micro arrays, are from the following species: *Fagus sylvatica*, *Lymantria dispar*, *Lymantria monarcha*, *Picea abies*, *Pinus pinaster*, *Pinus sylvestris*, *Quercus petrea* and *Quercus robur*. Data concerning provider, sequence information and annotation, as well as information related to production, such as quality of the purified PCR fragments, are stored in a database and will be accessible to all partners directly via the internet using www.evoltree.eu .

In addition to gene resources, also reference genomic DNA (gDNA) from various species will be available to the partners in order to guarantee uniform research material for mapping studies or marker evaluations. In a first step, plant material (leafs) from mapping pedigrees of oak and poplar were transferred to the ARC labs. Currently 1385 samples from the poplar cross 73028-62 x 101-74 from the gene bank at INRA Orleans and 413 genotypes of 3 different *Quercus robur x Quercus petraea* crosses (3P x A4, 11P x QS29, QS28 x QS21) are being validated and processed for DNA extraction. DNA extraction protocols are being developed for optimal DNA purity and amount, in order to be able to guarantee high quality DNA samples to the partners. Part of the leaf material is kept at room temperature as backup, whereas gDNA is aliquoted and stored for future requests. In the upcoming months, further plant material will be delivered by the evoltree gene banks as well as by the ISS. It is planned that several thousand samples from various species will be collected in Winter 2008/2009 from ISS, for future investigations on genetic diversity and interactions in the different forest ecosystems.

Activities planned within the Intensive Study Sites (ISS)

by Fabien Bachraty and Francois Lefevre

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As described in previous issues of this newsletter, the Network of Excellence evoltree has set up a series of Intensive Study Sites (ISS) as a shared infrastructure for multidisciplinary research. ISS are large scale ecosystem plots (a few thousands of hectares) where trees and selected associated species are mapped, genotyped and phenotyped.

The sites comprise entire portions of landscapes (agricultural land and woodlands) where trees are present in different configurations (from single trees to edges and woods).

A total of seven ISS have been selected, covering the main European forest types. The ISS Information System aims:

- to promote multidisciplinary research by sharing multiple information jointly related to the evoltree ISS
- to facilitate the access to datasets related to the evoltree ISS

- to preserve the knowledge of existing datasets
- to enhance understanding of the benefits of geographic information.

The evoltree ISS Information System is based on the GeoNetwork open source and allows easy sharing of geo-referenced thematic information between different organizations.
Geo-referenced datasets and maps (from single tree locations to satellite images) are efficient communication

Young spruces (*Picea abies)* establishment in dead trunk. Mixed old growth stand, Poland (Photo by Sascha Rösner | fotouristen.de).

tools for researchers, local managers and decision makers. The main goal of the evoltree ISS Information System is to improve accessibility to a broad variety of data and related metadata (data describing other data or a dataset) about ISS, obtained at different scales and from multidisciplinary sources, organized and documented in a standard and consistent way using ISO 19139 metadata and European INSPIRE directive.
The challenge is to enhance data sharing among the partners, to increase the collaborations and coordination efforts in collecting and exchanging information in the respect of intellectual property rights, to preserve data and spare resources.

The information system is based on metadata description that facilitate discovering, consultation and access to the available data related to each ISS. We decided to describe the available datasets (coming from heterogeneous supports: files, maps, databases, etc.) with the use of metadata that are stored in a centralised, customized, metadata management tool named Geonetwork. The information system was developed from June 2007 to August 2008. The tool is currently online (link[4]) and ISS correspondents started to describe their datasets in summer 2008.

The ISS Information System is expected to produce multiple benefits:

- at the individual level, scientists know the data they own and work with. Unfortunately, through time, this knowledge is progressively lost. Either people just forget they have the information about a particular subject or they do not remember where the data are located;
- the use of metadata is a guarantee that knowledge on the existing data is preserved;
- at the ISS level, the data description will allow a rapid survey of available data and it will help to avoid repeating the same work twice;
- at the Network level, it will be very interesting for each partner to discover the data that are available for each ISS and relevant for a particular research;
- it will be a good source of information coming from people working on the same research sites, and it will improve the capacity to conduct cross studies across sites, across disciplines and across partners;
- it is expected that the possibility to survery data available at the Network level will stimulate collaborations and facilitate decisions on joint acquisition of specific datasets like maps and others;
- finally, the global goal of the efforts is to enhance data integration and exploitation and to preserve the knowledge about existing data.

In addition, a web interface was developed to support sharing of the available data, presented through metadata descriptions. Even if data are not shared, different scientists within the network can contact data owners to obtain more details or to request access to the data themselves (contact details of data owners are displayed in association with metadata). The sharing interface allows the transfer and the update of datasets from the ISS to the central server and creates a link between the datasets and the corresponding metadata.
The ISS information system is part of the global evoltree information system. Direct links between the ISS information system and some other evoltree databases are planned, for example with the genebank database where genetic information on samples coming from the ISS (i.e. ISS trees or populations) will be stored.

ISS INFORMATION SYSTEM IN DETAIL

The information system consists of different parts:

- the Geonetwork tool used to describe the datasets with

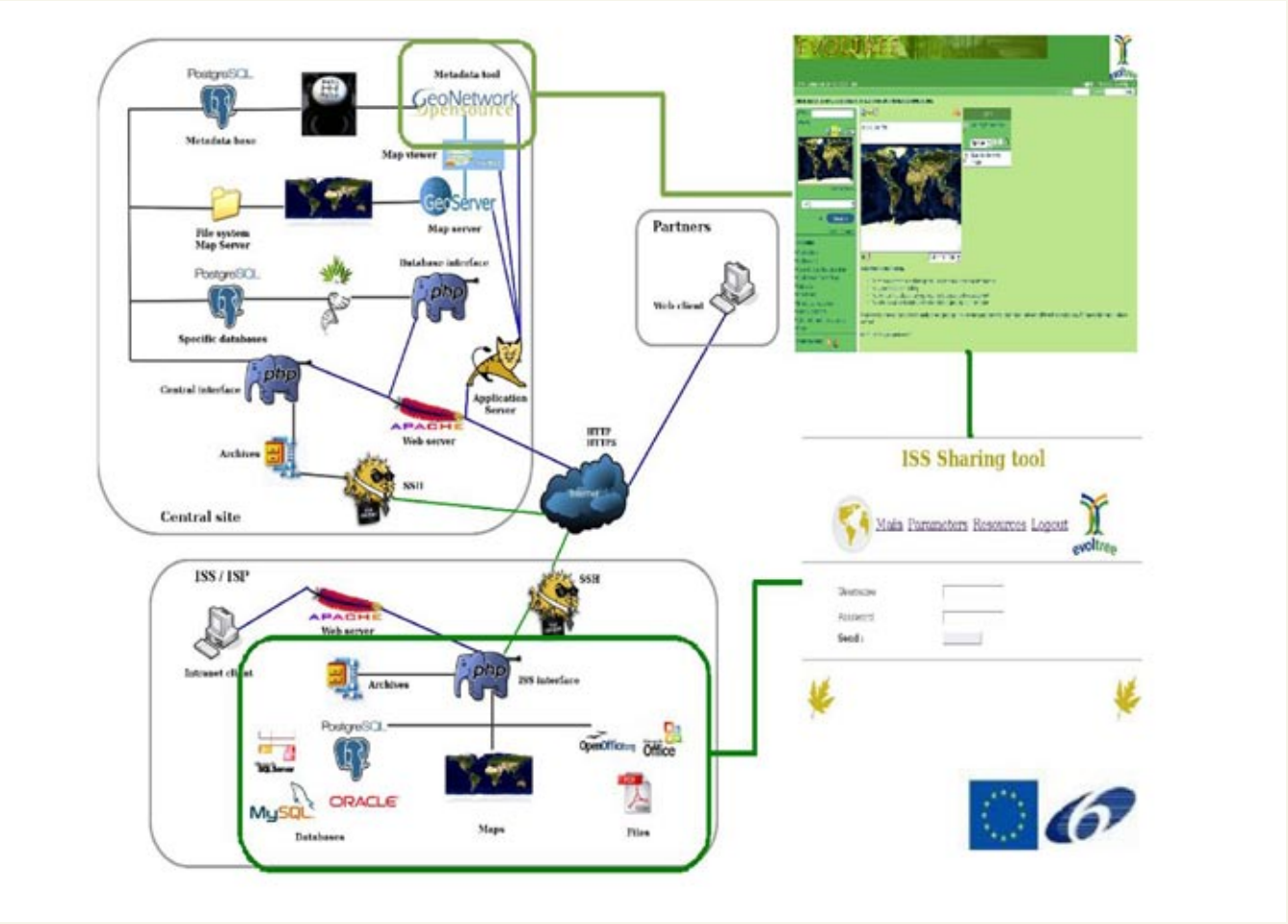

Figure 2. The Intensive Study Sites (ISS) information system.

metadata;

- the central interface used to centralize the dataset copies coming from the different ISS;
- the ISS interfaces used to define and maintain the shared datasets up to date;
- a PostgreSQL database where metadata are stored;
- a central file system that stores the datasets coming from the ISS into a central file system as archives, but these can also be integrated in a centralised database for database dump or in the central Geoserver for maps. When databases or maps are integrated into the central server, they can be directly consulted on the central server through the internet.

Otherwise, data must be downloaded and integrated locally to be used. The intermap tool is embedded within Geonetwork to display both integrated maps and metadata.

DATA DESCRIPTION AND SHARING

The description of the datasets is done through the evoltree Geonetwork tool. Once the data description is saved, a metadata is stored into the central server.

In a second step, the data sharing can be defined using the ISS interface. When a sharing is defined, an archive including a copy of the dataset is created (it can contain maps, database dumps and any other kind of files or directory). This archive is sent to the central server and can be updated whenever necessary through the ISS interfaces. Finally, in order to share the dataset copy present on the central server, the partners have to reference the data sharing within the metadata in order to link the data description and the data sharing. Refer to help documentation of Geonetwork and sharing interfaces for further details about the data sharing.

DATA DISCOVERING AND CONSULTATION

For discovering data, the partners just have to connect to the Geonetwork tool, then make a simple or an advanced search for available data through their metadata descriptions. If the user wants to download the dataset linked to the metadata, he just has to click on the link present in the metadata. Then the central interface will check his privileges, and if he is authorized to get the dataset, the central interface will invite the user to download the dataset archive or to consult it directly

online if it is integrated.

C RESEARCH HIGHLIGHTS

Achievements in comparative mapping in Fagaceaea (JERA1.3)

by Christophe Plomion

Chestnut (*Castanea*), beech (*Fagus*) and oak (*Quercus*) altogether belong to the angiosperm family Fagaceae, but show different relations: chestnut and oak belong to the subfamily Quercoideae, whereas beech is separated to the subfamily Fagoideae. Studies on detailed relations have been performed through morphological or genetic analyses.

However, researchers in JERA1.3 have been trying to find out whether the relations among species in the 3 genera could be elucidated also by comparative mapping of non-coding repetitive DNA regions, so-called SSRs. These regions, highly variable within single species, are also used for individual identification in forensic studies ('genetic fingerprinting').

An interdisciplinary study, performed by six evoltree partnering research groups, is focused on the challenge to explore whether common intraspecific patterns could be attributed to closely related species, and to what extent the differentiation into subfamilies has an influence in determining the observed patterns.

The project started by looking at the EST resources of *Quercus*. Within the map, putative SSR marker sites were located through special computerized procedures and tested in the lab. Besides being easy to use, EST banks indicate spatial location of transcriptional active sites within the genome.

A typical *Quercus*-fingerprint (length-pattern and positioning) was produced and a large-scale investigation, leading to a consensus SSR-based map of *Quercus*, is in progress.

The comparative mapping on *Castanea* and *Fagus*, based on *Quercus* ESTs, was performed in parallel. It revealed, as expected, a good transferability to *Castanea*, but a significant drop for *Fagus.*

The next objective of the investigation will be to localize the *Quercus*-homologue SSRs within mapping databases of *Fagus* and *Castanea*. To do this, a large EST bank has been established for *Fagus*, while the one for *Castanea* is underway within a NSF funded project.

Comparative mapping will help increasing knowledge on genome evolution at the macro-scale. Other consortia within the evoltree network are, in turn, working on comparative mapping approaches for other important tree families, such as Pinaceae and Salicaceae.

Update on full sequencing of chloroplast genomes (JERA1.2)

by Beppe Vendramin and Federico Sebastiani

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Chloroplasts are dynamic organelles of prokaryotic origin within the plant cell that house the photosynthetic apparatus. In addition to photosynthesis, other important metabolic activities take place within chloroplasts including the production of starch, certain amino acids and lipids, some of the colourful pigments in flowers, vitamins and several key steps of sulphur and nitrogen metabolism. Plastids have multiple copies of a circular, double-stranded DNA chromosome, each with a set of approximately 110 genes highly conserved in sequence and organization.

Some features of the plant organelle genome, such as the presence of two organelle genomes (compared to a single one – the mitochondrial genome - in most other eukaryotes) and the relatively high frequency of atypical modes of transmission (maternal, paternal or bi-parental inheritance, often distinct for mitochondrial - mtDNA and chloroplasts DNA - cpDNA) offer unique opportunities for phylogeographic studies and more in general for gene flow studies. In plants, as in animals, population studies and phylogeographic surveys have greatly benefited from polymerase chain reaction (PCR) approaches. The availability of completely sequenced chloroplast genomes in the 1980s, as well as the increase of available cpDNA sequences have allowed development of consensus (if not universal) primers of interest for intra-specific studies. On the other hand, a more ambitious cpDNA sequencing project involving some representatives from all major seed plant families would be very beneficial. For this reason, one important objective of evoltree is the sequencing of the chloroplast genomes of the three main species of the Fagaceae family (*Quercus robur, Fagus sylvatica* and *Castanea sativa*) and of *Pinus pinaster.*

The sequencing of the chloroplast genomes of *Quercus robur* and *Pinus pinaster* was recently completed while it is in progress for *Fagus sylvatica*. Together with the sequence of chloroplast genome of *Castanea sativa*, already available at the CNR-IGV lab in Florence, the plastid genomes of the three Fagaceae species will soon be released.

The same strategy was adopted for the different species: the genomes of chloroplasts was isolated by amplification of overlapping fragments with primers already available in the literature or designed within the project (*Grivet et al., Sebastiani unpublished).

A shotgun approach was adopted for the sequencing: the amplicons of each species, ranging from a few hundreds to 9 thousands base pairs, were mixed and sheared by nebulization, then cloned and sent to Genoscope for sequencing.

A total of 1,500 reads for each species was obtained. They were assembled using the software Phred/Phrap/Consed and using the *Castanea sativa* and *Pinus thunbergii* chloroplast genomes as reference for the assembly of the *Quercus robur* and *Pinus pinaster* cp-genomes, respectively. The gaps within these two genomes were amplified using specific primer pairs designed in the flanking regions and sequenced in the CNR-IGV lab.

The structure of *Quercus robur* genome turned out to be, as expected, similar (conserved) to the one of other angiosperm plastid genomes sequenced so far and, in particular, to that of *Castanea sativa*. In fact the size of the oak cp-genome is about 161,000 bp, slightly different from that of chestnut (158,998 bp). The main differences in their sizes is due to single nucleotide polymorphisms and indels.

The chloroplast genome of *Pinus* spp. is different in both size and structure from that of the angiosperm species. The size of *Pinus pinaster* chloroplast genome is smaller than that of the three Fagaceae species (about 120,300 bp) and similar to that of *Pinus thunbergii* (119,707 bp).

Large woody debris in an old-growth deciduous forest, Poland (Photo by Sascha Rösner | fotouristen.de).

Moreover the *Pinus pinaster* cp-genome lacks one of the inverted repeat element, as already observed in other conifer species.

*Grivet D, Heinze B, Vendramin GG and RJ Petit (2001) Genome walking with consensus primers: application to the large single copy region of chloroplast DNA. Molecular Ecology Notes 1: 345-349.

BEECH - Community structure and dynamics within beech stands - Phase I: Intraspecific processes within *Fagus sylvatica* populations (JERA3)

by Igor Chybicki and Jaroslaw Burczyk

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Common beech (*Fagus sylvatica* L.) is one of a few keystone tree species that covers a large portion of Europe. Forests dominated by *Fagus* are found over a remarkably wide range of environments. The structuring role of beech in forest ecosystems is well recognized. However, the mechanisms underlying the ecosystems' stability, and their potential for adaptation to global changes, are only starting to be understood. Gene flow through pollen and seeds seems to be one of the mechanisms enabling adaptation of populations to environmental changes. The initial phase of the BEECH project was focused on intraspecific processes, including the patterns of gene flow through pollen and seed dispersal as observed at the stage of naturally established seedlings. The goals were to estimate distances and rates (dispersal curves) for effective pollen and seed dispersal, and to draw some conclusions on the potential for long distance gene flow and its role for adaptation to global changes.

The four partners engaged in the project are: Kazimierz Wielki University of Bydgoszcz (Poland), INRA-Avignon (France), University of Gottingen (Germany) and Alterra (Netherlands). A total of 7 study sites were selected across 4 locations: ISS Blizyn (Poland, 2 stands), ISS Ventoux (France, 2 stands), ISS Soling (Germany, 2 stands) and ISP

A natural beech stand from the Dalejow sample plot, within the Intensive Study Site of Blizyn, Poland (Photo by Jaroslaw Burczyk, UKW).

Veluwe (Netherlands, 1 stand). Each stand represented a different type of beech forest, ranging from pure beech stands, through mixed beech-silver fir or beech-Norway spruce stands (various beech densities), to stands with scattered beech trees. More than 1,000 adult trees and 3,000 seedlings have been sampled and mapped. Genotyping of all sampled individuals was done based on a set of microsatellite markers (common set: fs1-15, sfc0018, sfc0161, sfc1063, sfc1143, mfs11; additional optional loci: sfc0195, sfc1105; *Pastorelli et al. 2003, **Asuka et al. 2004, ***Vornam et al. 2004). The collaboration between partners was tightened through several visits among partners (facilitated by evoltree activity IA4. - Mobility), which enabled to standardize and unify laboratory procedures, and also (more importantly) i to mprove and update analytical methods for studying gene flow based on naturally established seedlings. Trial analyses performed to date, based on the data available from four populations, indicate that most features of effective gene flow are shared among different types of beech forests, including low average seed dispersal (up to 10 meters within a site), moderate to high average pollen dispersal distances (20-70 meters within a site), low to moderate seed immigration (the immigration of seeds from outside the study plot varied from 0.0% to 25%, and was higher in mountainous than lowland ecosystems (a slope plays a role as dispersal factor) but relatively high pollen immigration (above 50%) and fat-tailed pollen dispersal kernels.

In the mean time two research teams, managed by Francis Martin (INRA-Nancy - France) and Jaroslaw Burczyk (Kazimierz Wielki University of Bydgoszcz – Poland), were awarded a research grant under the INRA-MSHE (MSHE - Ministry of Science and Higher Education, Poland) collaborative program (see note on the Joint Scientific Project (JSP) MYCBEECH on: "Genetic processes within populations of *Fagus sylvatica* and its ectomycorrhizal fungi as mechanisms for ecosystem adaptation to global changes" in the section Announcements).

*Pastorelli R, Smulders MJM, Van't Westende WPC, Vosman B, Giannini

Sequencing of chloroplast genome of beech (*Fagus sylvatica)* is still in progress. Beech leaf (Photo by Sascha Rösner | fotouristen.de).

R, Vetori C and GG Vendramin (2003) Characterization of microsatellite markers in *Fagus sylvatica* L. and *Fagus orientalis* Lipsky. Molecular Ecology 3: 76-78.
**Asuka Y, Tomaru N, Nisimura N, Tsumura Y and S Yamamuto (2004) Heterogeneous genetic structure in a *Fagus crenata* population in an old growth beech forest revealed by microsatellite markers. Mol Ecol 13: 1241-1250.
***Vornam B, Decarli N and O Gailing (2004) Spatial Distribution of Genetic Variation in a Natural Beech Stand (*Fagus sylvatica* L.) based on Microsatellite Markers. Conservation Genetics 5: 561-570.

COMMUNI-TREE - Tree community genetics. Understanding diversity of associated mycorrhiza and insects (JERA3)

by M.J.M. René Smulders

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Interactions between species can be very complex. Trees, as keystone species of ecosystems, can support large numbers of organisms, and some traits of trees were shown to have major influence on insect communities (*Whitham et al. 2006). Studying the genetic diversity of tree populations and the effects of specific tree traits (genes) on associated communities is therefore an approach to understanding ecosystem community diversity. This knowledge may significantly influence our insight in optimal designs for reforestation and nature restoration.

Within evoltree, twelve partners have started a project on community genetics of poplar and oak. The aim is to characterise the effects of poplar and oak trees on the genetic structure and (functional) diversity of two groups of associated organisms, namely mycorrhizal fungi and tree-related leaf-eating, leaf-mining, and gall-inducing insect species. The effect of the tree species is approached in three ways: i) the effect of the level of genetic diversity within a stand, ii) the effect of genetic distance between genotypes, iii) and the effect of particular genotypes per se. The experimental plots have been established in 2008 (see Figure 3).

Detection of the effect of genetic diversity of oak tree seedlings on the species diversity of associated insects will be studied in common gardens where different levels of genetic diversity will be compared, from one plot of seedlings from a single half-sib family to one plot with a mixture of seedlings from 16 families. All plots contain 64 individuals. A sample of 64 seedlings naturally regenerating in the same stand will provide a control plot to estimate the local pool of associated species. This experiment is carried out in France (ISS Landes), UK, and Hungary. Detection of the effects of genetic diversity of poplar host trees on species diversity of insects will be studied in a similar design, but using collections of cuttings of black poplar clones that vary in number of genotypes. These experimental plots have been established in France (ISS Loire), Germany, Hungary, Italy, and Poland.

The effect of genetic distance between Oak tree seedlings on the species diversity of associated insects is studied in the same common garden design in the same stands (ISP) as above but with clones or families that will have been previously obtained from seeds of distant mother trees.

To determine the effect of genotype on associated fungi and insects we established collections of poplar cuttings consisting of 16 genotypes, including the parents of existing segregating populations, replicated at four sites (Hungary, Italy, Poland, and Germany). Differences in associated organisms that are specific for certain genotypes rather than sites will enable mapping the traits in existing segregating populations, as a first step to identify candidate genes for the traits that influence the interactions between species. Genotype and site effects can be disentangled also in the data on associated organisms of mature *Pinus nigra 'Italica'* trees growing near these experimental plots.

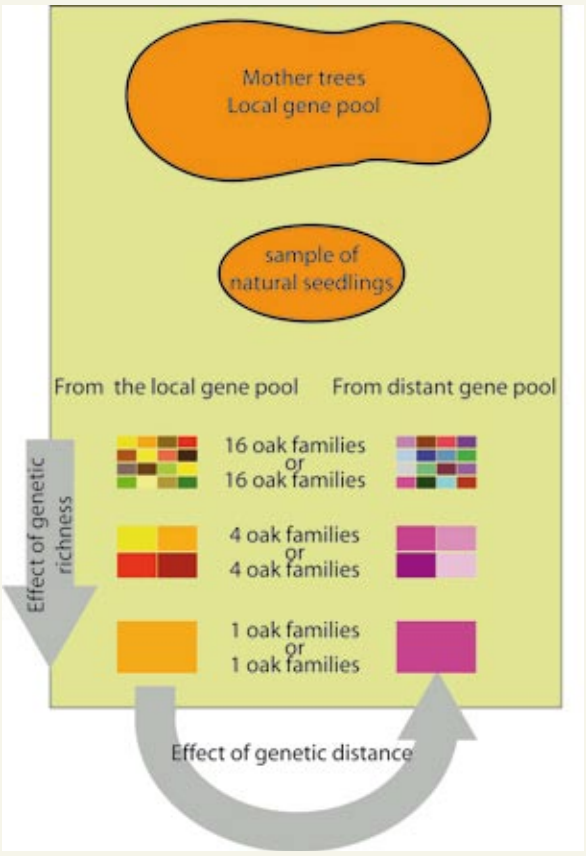

Figure 3. Oak experimental plots (graphic by Herve Jactel).