

The sediments studied consisted of olive grey to brown, muddy silt occasionally mottled and faintly laminated. Black specks, shell fragments and macroalgae plant debris characterised the sediments from the shelf trough and fjords. The chronology was based on ^{210}Pb and ^{137}Cs measurements and Accelerator Mass Spectrometry ^{14}C dating of shells and plant remains.

The distribution of POM in environments ranging from coastal water to deep-sea showed regular changes controlled by the distance from the shore. Phytoclasts represented the major component in the total POM assemblage in fjord and shelf trough settings. In the fjords, the phytoclasts were dominated by leaf and membranous tissue characterised by well-preserved clasts of variable size (up to 400 μm in diameter). At the shelf trough site, wood and charcoal dominated the phytoclast assemblages; leaf and membranous tissue represented a minor component of the assemblages and were characterised by small clasts (15–30 μm in diameter). Phytoclasts were rare to absent in the sediments from the North Atlantic deep-water site.

The palynomorph assemblages from the fjords and shelf trough site were dominated by dinoflagellate cysts and acritarchs and showed a similar distribution pattern in the sites. The palynomorph assemblages from the North Atlantic deep-water site consisted of a selection of organic material, which could result from transport or grading. Dinoflagellate cysts were the dominant component of the total POM and represented 55–65% of the assemblages.

Prasinophyte algae occurred in all study sites ($\leq 4\%$ of the total POM). According to TYSON (1995), prasinophytes represent one of the major phytoplankton component in cold waters at high latitudes together with diatoms; however, they can be abundant in the water column but scarce in the sediments. In the study sites, the highest percentages of prasinophytes were recorded from the shelf trough site off West Greenland and Igaliku Fjord, South Greenland.

As a result, the relative abundance of foraminiferal linings, dinoflagellate cysts, acritarchs and other marine algae in the total POM assemblage increased with the distance from the shore and can thus be used as a proxy for the distance from the shore in past depositional environments at the high latitudes.

TYSON, R. V., 1995. *Sedimentary organic matter. Organic facies and palynofacies*. Chapman & Hall, London, 615 pp.

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DYNAMIC OF ECOSYSTEMS: PALYNOLOGY AND GENETICS

Reconstructing the past: the evolving roles of the pollen record and extant and fossil DNA

Lascoux, M.

Program in Conservation Biology & Genetics, Evolutionary Biology Centre Uppsala University, Norbyngen 18D 752 36 UPPSALA, Sweden.

Data on the genetic structure of plant populations at the continental scale have accumulated dramatically over the last decade. However, our ability to make inferences on the impact of the last ice age still crucially depends on the availability of informative pollen fossil data. When the pollen record is non-informative, inferences on past history are difficult to draw from the sole genetic data. On the other hand, when the fossil record is informative, new approaches, based on the coalescent process, allow the testing of well-defined historical hypotheses and the estimation of demographic parameters. Examples will be provided. We will also discuss the promises and limitations of ancient DNA in the reconstruction of past history.

Fossil and genetic data outline the late-Quaternary history of *Fagus* in Europe

Magri, D.¹ & FOSSILVA *Fagus* Group.²

¹ Dipartimento di Biologia Vegetale, Università "La Sapienza", P.le Aldo Moro, 00185 Roma (Italy). ² EU FOSSILVA Project *Fagus* Group members contributing to this paper: COMPS, B. (Bordeaux, France), DE BEAULIEU, J.-L. (Marseille, France), DUPANLOUP, I. (Lausanne, Switzerland), GEBUREK, T. (Wien, Austria), GÓMÖRY, D. (Zvolen, Slovakia), LATALOWA, M. (Gdansk, Poland), LITT, T. (Bonn, Germany), PAULE, L. (Zvolen, Slovakia), PETTI, R. (Bordeaux, France), ROURE, J.M. (Barcelona, Spain), TANTAU, I. (Cluj-Napoca, Romania), VAN DER KNAAP, W. O. (Bern, Switzerland), VENDRAMIN, G. G. (Firenze, Italy).

The European vegetation of the present interglacial is characterized by a widespread diffusion of *Fagus sylvatica*, extending from the Cordillera Cantabrica to the Carpathians and from Sicily to northern Sweden. Such extensive geographical distribution is very recent, dating back to the last few millennia, while a continuous presence of beech during the last glacial-interglacial cycle can be traced only in restricted geographical areas. These observations stimulate the discussion on the timing and mode of the postglacial spread of *Fagus* populations and on the influence that the past beech distribution may have had on its modern genetic characters.

For that purpose, a group of geneticists and palaeobotanists working within the EU FOSSILVA project have joined their efforts in carrying out new analyses and collecting unpublished and published data from hundreds of sites in Europe. Four data-sets have been compiled, including fossil pollen and plant macrofossils for the palaeobotanical side, and chloroplast and nuclear markers for the genetic side.

The fossil data indicate that beech survived at multiple sites during the last glacial period. However, contrary to what is generally thought, the refuge areas located in the Italian, Balkan and Iberian peninsulas were not source areas for the postglacial colonization of central Europe by beech. In fact, the patterns of migration indicate that *Fagus* spread from Slovenia and from other minor refuge areas in mountain regions of central Europe. Beech migrated along mountain chains until ca. 4 kyr BP, when it reached the plains of central-northern Europe.

The modern genetic variability, evaluated by means of markers with different modes of inheritance and mutation rates, processed by various numerical methods, supports the general pattern shown by the fossil data, and in a number of cases defines with better precision the postglacial migration routes followed by beech.

The multidisciplinary analyses of European beech offer a new scenario for the location of glacial refugia and the postglacial routes of colonization for beech, at more northern latitudes than previously thought, and at the same time open new methodological perspectives for a better understanding of: i) type and quality of fossil and genetic data best suited to detect areas of long-term persistence of deciduous trees, ii) geographical and biological barriers to colonization, iii) relation between the postglacial rates of spread and the modern genetic structure and patterns of diversity, iv) influence of refugia in protecting or in shaping biodiversity, v) importance of the time factor and of climate changes over multiple glacial-interglacial cycles in determining genetic divergence.

The FOSSILVA project (dynamic of forest tree biodiversity: linking genetic, palaeogenetic and plant historical approaches): an overview

de Beaulieu, J. L.¹ & Fossilva members²

¹ Institut Méditerranéen d'Ecologie et de Paléocécologie, Université d'Aix-Marseille III, 13397, Marseille cedex 20, France. ² Fossilva members: Ammann B., Anzidei M., Andrieu V., Begeot C., Bertel L., Burban C., Carnevale S., Cheddadi R., Cherisey H., Coldea G., Davis B., Deguilloux M.-F., Dubois J.-M., Dumas S., Farcas S., Follieri M., Gielly L., Gómory D., Guibal F., Homb B., Huerne R. ter, Knaap W. O. van der, Kubitz B., Lambert E., Latalowa M., Leeuwen J. F. N. van, Liepelt S., Litt T., Magri D., Mateus J., Miquel C., Miramont C., Pantaleone Cano J., Pastorelli R., Perez-Obiol R., Petit R., Popescu F., Queiros P., Roure Nolla J. M., Sadori L., Sperisen C., Taberlet P., Tantau I., Vendramin G., Wicha S., Yli E.-I., Ziegenhagen B.

The first aim of FOSSILVA was the study of past biodiversity in fossil tree material across a network covering south-western and central Europe: a better knowledge of the historical parameters behind the present day

fragmented forests may help to define their origins and affinities and thus will contribute to improve their management. The project involved for the first time an equal number of partners from two communities: palaeobotanists working on natural archives (lake sediments, peat bogs, buried trunks and old timbers) and plant population geneticists. The potential for the recovery and study of fossil DNA in different tree remains has been explored. The subsequent goal was a comparison of migration routes inferred from palaeo data (pollen, plant macrofossils) with intraspecific genetic data based on both fossil and modern DNA data to help understand the origin of the modern forests and their rate of disturbance; for this task the study had been targeted on a limited number of forest trees: two broad-leaved species (*Fagus sylvatica*: beech and *Quercus* spp.: white oaks) and four conifers (*Abies* spp.: firs, *Pinus sylvestris*: Scots pine, *Pinus pinaster*: maritime pine, and *Picea Abies*: spruce). They have been selected according to the following criteria: 1/ their present day genetic diversity must have been already at least partly explored, 2/ consistent hypotheses on their Holocene setting have already been formulated that needed improvement and validation. The strategy is developed in order to: 1. The project has contributed to the improvement of the European Pollen database and in the launching of a macrofossil database, leading to a better understanding of the postglacial migrations of the selected trees from their glacial refuges; the comparison with the phylogeographical approach led to consistent progress on the understanding of their present day setting (cf other presentations in this session). The extraction of fossil DNA from plant material appeared extremely difficult and our attempts suggest that the conservation of DNA is very erratic. A few positive results illustrate the potential and the limits of this approach.

Ancient DNA in pollen—genetic analysis in two Holocene pine populations from Sweden

Parducci, L.¹; Suyama, Y.²; Lascoux, M.³ & Bennett, K. D.¹

¹ Palaeobiology, Department of Earth Sciences, Uppsala University, Villavägen 16, S-752 36, Sweden.

² Graduate School of Agricultural Science, Tohoku University, Kawatabi, Naruko, Miyagi 989-6711, Japan.

³ Department of Conservation Biology and Genetics (EBC), Uppsala Univ., Norbyvägen 18/D, S-752 36, Sweden.

Recent advances in molecular biology offer now excellent tools to obtain DNA information from well-preserved fossil material and the discovery that ancient DNA can be amplified by polymerase chain reaction (PCR) has added a direct temporal dimension to many evolutionary studies. By using a method we recently developed for the analysis of DNA in fossil pollen, we examined the chloroplast DNA sequence variation among fossil pollen grains of Scots pine (*Pinus sylvestris* L.) incorporated in a Holocene sediment core collected in central Sweden. DNA sequences data obtained from fossil (100 and 10,000 cal. BP) and modern samples revealed a number of different haplotypes and their relationships were visualised by a statistical parsimony network analysis. Results from this study establish a first genetic link between modern and fossil DNA samples and provide unique genetic information useful for understanding the origin and the evolution of Scots pine ancient populations.

Postglacial migration and ice age refugia of *Picea glauca* (white spruce) in North America: evidence from chloroplast DNA

Anderson, L. L.; Hu, F. S. & Paige, K. N.

Program in Ecology and Evolutionary Biology, School of Integrative Biology,
University of Illinois, Champaign/Urbana (United States).

Two long-standing hypotheses exist concerning the ice-age refugia and postglacial migration of white spruce in North America. The first proposes that refuge populations were restricted to areas south of the Laurentide ice sheet and that white spruce spread extremely fast northward following the end of the last glaciation. The second proposes that additional refugia existed in unglaciated areas of Beringia and Canada during the last glacial maximum and that the postglacial spread rates were much slower. We are using a suite of

chloroplast markers from modern populations and macrofossils preserved in lake sediments to test these hypotheses.

Analyses of modern foliar samples of white spruce across North America revealed three polymorphic regions within the chloroplast genome. DNA sequencing of 200 individuals from 18 populations uncovered 16 haplotypes. Several of these haplotypes are restricted to local regions throughout the sampling area, including Alaska, and have a significantly nonrandom geographic distribution. This nonrandom distribution is inconsistent with the pattern expected from a single northward migration. Thus our data suggest the existence of multiple refugia. Additional sampling of modern samples and chloroplast DNA analysis of macrofossils preserved in sediments should substantiate the existence and location of refugial populations.

Migration routes and refugia of *Podocarpus* in Brazil during the Late Quaternary: Some preliminary results

Ledru, M. P.¹; Salatino, M. L. F.²; Ceccantini, G.²; Salatino, A.² & Witovisk, L. G.²

1 IRD, UR 055, 32 av. Henri Varagnat 93143 Bondy cedex France, and IRD/ISEM/Univ,
Montpellier 2 CP 61, Place Eugène Bataillon 34095 Montpellier cedex France.

2 Universidade de São Paulo Instituto de Biociências, rua do Matão, São Paulo SP Brazil.

Podocarpus selowii K. and *Podocarpus lambertii* K. (Podocarpaceae) represent two Brazilian endemic species of this southern hemisphere conifer. They occur today in southern Brazil as widespread populations associated with *Araucaria angustifolia* rain forest and in northeastern and central Brazil as habitat-restricted populations. Systematic separates these two species on the base on foliar morphology mainly, long/wide versus short/narrow. However, we observed frequently the presence of these two types of leaves on the same tree and therefore we decided to collect and analyse both species without discrimination. Also when we refer to pollen data, determination stops at a genus level; then we will speak of *Podocarpus sensus largo*. *Podocarpus* proved to be able to respond rapidly to abrupt climatic change during the deglaciation and expanded widely from sites where it occurs as refugia today; also it proved to disappear from our pollen records during the last glacial maximum from areas where it is widespread today. In order to elucidate the biogeographic history of *Podocarpus* populations in the tropical lowlands of South America we carried out a phylogenetic analysis of chloroplast DNA from 26 populations distributed between 30°S latitude and the equator, from sea level to 1800 m elevation. This molecular approach should provide new insights into the location of Atlantic rain forest refugia and routes of migration during the climatic oscillation of the Quaternary. This approach is also important to conservation efforts by providing tool for measuring and managing genetic diversity in tropical forests and for investigating the processes that influence it.

Out of refugia: mapping the invasion routes of trees using molecular markers

Petit, R. J.¹ & Vendramin, G. G.²

¹ UMR Biodiversity, Genes and Ecosystems, INRA, 33612 Cestas (France).

² CNR, Plant Genetics Institute, 50019 Firenze (Italy).

In combination with fossils, molecular markers can allow fine reconstruction of past tree refugia and subsequent expansion routes. Markers may be especially useful to reconstruct movements in species that migrated fast along a broad front, which corresponds precisely to the case when fossils will provide little resolution. Beyond the fascination of reconstructing exact movements of past tree populations, the knowledge of the spatio-temporal dynamics of forest trees could tell us a great deal about past environments and climate.

Such reconstructions may at first seem particularly easy: A given molecular variant is mapped within the species range and a comparison is made with maps of pollen or other fossils. Fossil data provide the starting point(s) of the expansion and hence the direction of colonisation (generally northwards in the Northern

hemisphere, southwards in the Southern Hemisphere). In fact, several issues request careful examination of the data and many difficulties quickly arise.

First, the quality of the reconstruction will depend on the sampling intensity and the presence of populations in the intervening regions between refugia and current distribution of trees. One may have to reconstruct colonisation routes with an incomplete picture.

Second, appropriate fossil data may be limited or completely lacking (as in insect-pollinated taxa) or an independent confirmation of the fossil data may be desirable. In principle, if sufficient mutations have occurred during the right time lapse, a phylogenetic analysis of range expansion is possible, allowing the stepwise identification of more and more derived population and hence the identification of the polarity of the movements. However, this requests high mutation rates since colonisation was generally a rapid event. Although maternally-inherited markers may seem *a priori* ideally suited to reconstruct colonisation routes, since they keep a record of the movements through seeds uncomplicated by pollen gene flow, they evolve at a reduced pace, so such an analysis is rarely possible.

Third, even if the reduced evolutionary rate of chloroplast or mitochondrial DNA have been known for some time, it seems that this has been frequently overlooked, resulting in the faulty attribution of colonisation routes to the postglacial period, as in oaks and in beech. It now appears that many phylogeographic analyses reveal instead invasions predating the Last Glacial Maximum.

Other potential pitfalls include simplistic assumption (i) of northwards movement, (ii) of systematic decrease of diversity during invasion, and (iii) of the role of barriers played by mountainous areas. They will be illustrated with case studies. Over-reliance on common expectations such as northward movements in the Northern Hemisphere would have been misleading for most temperate tree taxa in Europe due to extensive westward movements. The fact that genetic diversity is much well preserved than commonly expected during colonisation has already been discussed (e.g. Petit et al. 2001) and could indeed result in the misinterpretation of genetic data. Another scenario, which is not always borne out, is the role of potential barriers played by mountain ranges. In fact, depending on the species, mountains may have played just the opposite role, that of preferred corridors, along with large rivers or coastal habitats now lying under the Sea.

As these limitations get better appreciated and reconstructions improve, it is becoming increasingly clear that the climate prevailing at the time of colonisation and the nature of the recipient community can no longer be ignored. Such improved reconstructions of colonisation routes should now allow the testing of concepts of invasion theory, resulting in an improved understanding of past natural invasions and better comparisons with human-induced ones.

PETTIT, R. J., BIALOZYT, R., BREWER, S., CHEDDADI, R. & COMPS, B. 2001. From spatial patterns of genetic diversity to postglacial migration processes in forest trees. In: Silvertown, J. and Antonovics, J. (éds.). *Integrating ecology and evolution in a spatial context*. Oxford, Blackwell Science, pp. 295-318.

Pollen as main agent of gene flow in wind-pollinated trees

Liepert, S. & Ziegenhagen, B.

Philipps-University of Marburg, Biology Department, Conservation Biology Division,
Karl-von-Frisch-Str., 35032 Marburg (Germany).

Molecular markers are useful tools to study the spatial distribution of genetic variation. The resulting patterns are used to reconstruct past processes such as migration and gene flow. Genetic markers from the organelle DNA can be especially suited to reconstruct these processes, since they are uniparentally inherited in many species. The conifers of the *Pinaceae* family are a special case, since their organelle genomes exhibit contrasting modes of uniparental inheritance, i.e. mitochondrial DNA is inherited maternally and chloroplast DNA is inherited paternally.

The conifer *Abies alba* (Mill.) was used as a model species to analyze the maternal as well as the paternal aspect of range-wide distribution of genetic diversity (LIEPELT *et al.* 2002). Two DNA markers with contrasting modes of inheritance were applied to 100 populations covering the entire distribution range of *A. alba*. The markers exhibited each two highly conserved alleles based on an insertion/deletion of 80 bp in the fourth intron of the mitochondrial *nad5* gene and on a synonymous substitution in the chloroplast *psbC* gene. The populations and allele frequencies of both markers were mapped geographically and clines were calculated. The

geographical distribution of the maternally inherited mitochondrial variation supported the existence of at least two refugia. The maternal lineages re-colonised the range remaining largely separated, which resulted in a steep and narrow allele frequency cline.

In contrast, most of the populations were mixed for the two chloroplast *psbC* alleles. Geostatistical analysis confirmed a significant and range-wide east-west cline of allele frequencies for the chloroplast marker. Our results strongly suggest that an exchange of genetic information between refugia by range-wide paternal introgression occurred in the present interglacial. Due to life-history traits that encumber gene flow, *A. alba* was proposed as a model for wind-pollinated trees in general.

In a second study, contrasting organelle DNA markers were applied to study genetic relationships and gene flow among eight Mediterranean *Abies* species. The maternal lineages again exhibited a sharp geographic separation while their is evidence that paternal lineages are shared among maternal lineages and thus also among species. This led to the hypothesis that Mediterranean firs share a gene pool that is maintained by pollen-mediated gene flow.

LIEPELT, S., BIALOZYT, R. & ZIEGENHAGEN, B. 2002. Wind-dispersed pollen mediates postglacial gene flow among refugia. *Proceedings of the National Academy of Sciences, USA* 99: 14590-14594.

Impact of the last glacial period environment on the genetic diversity of *Pinus*

Cheddadi, R.¹; Vendramin, G.²; Litt, T.³ & Dubois, J. M.¹

¹ Université Montpellier II, Institut des Sciences de l'Évolution, UMR 5554, 34095 Montpellier, France.

² Istituto di Genetica Vegetale, Via Madonna del Piano, Edificio D, 50019 Sesto Fiorentino, Firenze, Italy.

³ University of Bonn, Institute of Paleontology, Nussallee 8, D-53115, Bonn, Germany.

During the last glacial period the temperate European tree species were constrained under severe climatic conditions to survive in a few restricted refugia. Unlike deciduous trees, many conifers such as pines may stand very low winter temperature and low annual precipitation and therefore they may have had more potential refugial areas during the last glacial period. Combined paleoenvironmental and genetic studies have shown that the location of the glacial refugia and the post-glacial migrational paths taken by many European temperate species, have played a major role in shaping their modern distributions of genetic diversity (Petit *et al.*, 2003). Here we discuss the case of pine, a conifer species. The modern distribution range of pine shows that its climatic amplitude in terms of annual precipitation and winter temperature is between 400 and 900 mm/year, and -18°C to +2°C, respectively. The European Pollen Database gathers a network of fossil pollen data, which allowed us to reconstruct the climate of the last glacial period. Quantified climatic parameters for the period spanning the last glacial show that several areas in Europe may have played the role of refugia where pine populations had some chance of survival.

A genetic survey at the European scale, using mitochondrial DNA, depicts three different haplotypes for *Pinus sylvestris*. Two distinct ones restricted separately to the northern Iberian peninsula and the Alpine region and the third dominates most of the remaining distribution range of pine in Europe.

Several paleobotanical studies in Europe show that pine is frequent among the pioneer tree species recorded in the fossil pollen and plant remains analyses. Such an early spread throughout Europe at the end of the last glacial period may be explained by the presence of a good number of scattered refugia. The present study combining paleoenvironmental and genetic data tend to indicate that the today dominant pine haplotype has persisted in several and separate refugia most probably without mutating during the last glacial period and that the high migrational rate of the species from the numerous scattered refugia may explain its widespread and dominance throughout Europe.

PETTIT, R., *et al.* 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300: 1563-1565.