DYNAMICS AND CONSERVATION OF GENETIC DIVERSITY IN FOREST ECOSYSTEMS

Conference sponsored by:

EUROPEAN COMMISSION (DG Research, Programme “Quality of Life and Management of Living Resources”) Project QLAM-2001-00274
INRA (Institut National de la Recherche Agronomique, Département « Forêt et Milieux Naturels »)
IPGRI-EUFORGEN (European Forest Genetic Resources Programme)
IUFRO Working Party 2.04.01 “Population, Ecological and Conservation Genetics”
ECOFOR (GIP “Ecosystèmes Forestiers”)
DERF (Direction de l’Espace Rural et de la Forêt, Ministère de l’Agriculture et de la Pêche)
Ville de STRASBOURG

Organizing Committee:

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Outi Savolainen (IUFRO, University of Oulu, Finland)
Josef Turok (IPGRI, EUFORGEN, Rome, Italy)

Scientific Committee:

Coordinators of EU supported projects in framework 4 and 5 in the field of Population Genetics of Forest Trees and chairs of EUFORGEN networks

Michel Bariteau, Jacques Louis De Beaulieu, Eric Collin, Barbara van Dam, Luis Gil, Hans Hattemer, Koen Kramer, Antoine Kremer, Csaba Matyas, Hans Mühs, Gerhard Müller Starck, Ladislav Paule, Rémy Petit, Christophe Plomion, Mari Rusanen, Outi Savolainen, Florian Scholz, Janet Stewart, Ludwig Triest, Josef Turok, Maria Carolina Varela, Fiorella Villani, Sven de Vries, Monica Van Wordragen
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<td>08:40 - 08:50</td>
<td>Norbert Winkler, Scientific officer, Directorate E - Life Sciences, DG Research</td>
<td>Research support in genetic diversity of European trees in framework programmes 4 and 5</td>
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<td>08:50 - 09:00</td>
<td>Pierre Mathy, Head of Unit &quot;Biodiversity and Marine Ecosystems&quot;, Directorate I DG Research</td>
<td>Perspectives for biodiversity and ecosystem research in framework programme 6</td>
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<td>09:00 - 09:10</td>
<td>Eric Teissier Du Cros, IUFRO-Vice President-Science, Chairman of the committee of forest genetic Resources IUFRO</td>
<td>Its contribution to management and conservation of forest genetic resources.</td>
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<td>09:10 - 10:50</td>
<td>Phillip Hedrick Recent Developments in Conservation Genetics</td>
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<td>10:20 - 10:50</td>
<td>Peter Smouse Measuring pollen flow in forest trees: A comparison of alternative approaches</td>
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<td>10:50 - 11:25</td>
<td>Loren Rieseberg Hybridization and ecological divergence in annual sunflowers</td>
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<td>11:25 - 12:00</td>
<td>Outi Savolainen Genetic variation in timing growth in Scots Pine and adaptation to climate change</td>
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<td>12:00 - 12:35</td>
<td>Robert Latta Processes shaping genetic variation across landscapes</td>
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<td>12:35 - 14:00</td>
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<td>14:00 - 14:20</td>
<td>A1-1 Victoria Sork et al. Contemporary pollen movement within and among wind- and insect-pollinated tree species</td>
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<td>14:40 - 15:00</td>
<td>A1-3 Felix Gugerli et al Biparentally, maternally, and paternally inherited molecular markers elucidate population processes on a small scale within a Norway spruce stand</td>
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<td>15:00 - 15:20</td>
<td>A1-4 Cecile Bacles et al Comparing the effects of habitat fragmentation on gene flow for rowan and ash in a Scottish landscape.</td>
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<td>A1-6 Myriam Heuertz et al Estimating pollen versus seed dispersal from spatial genetic structure in the common ash</td>
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<td>16:10 - 16:30</td>
<td>A1-7 Marie Elise Morand Prieur et al Limited seed dispersal in a partially scattered tree species, Fraxinus excelsior L., as revealed by parentage analysis using microsatellites</td>
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<td>16:30 - 16:50</td>
<td>A1-8 Phillip England et al Genetic structure at different spatial scales in an endangered fire-dependent plant.</td>
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<td>17:10 - 17:30</td>
<td>A3-1 Gerhard Müller-Starck et al Biodiversity in Alpine forest ecosystems: analysis and management (BAFE CT96-1949)</td>
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<td>17:30 - 18:10</td>
<td>A3-2 Michel Bariteau et al. Neutral and adaptive genetic diversity of Mediterranean Conifers</td>
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<td>18:10 - 18:30</td>
<td>A3-3 Olivier Brendel et al Genetic determination of leaf carbon isotope composition for a pedunculate oak family</td>
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<td>18:30 - 18:50</td>
<td>A3-4 Marco Lauteri et al Genetic variation in carbon isotope discrimination in six European populations of Castanea sativa Mill. originating from contrasting localities</td>
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<td>18:50 - 19:10</td>
<td>A3-5 Christian Lexer et al Combining QTL mapping with selection studies in the wild: a feasible way to identify adaptive genetic variation in outbreeding forest trees?</td>
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<td>19:10 - 19:30</td>
<td>A3-6 Christine Dillmann et al Genetic variability in the course of adaptation towards a far optimum</td>
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<td>19:30 - 19:50</td>
<td>A3-7 Pauline Gere et al. Genetic diversity and differentiation of neutral genes linked to QTLs controlling a polygenic adaptive trait under stabilizing and diversifying selection</td>
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<td>19:50 - 20:10</td>
<td>A3-8 Santiago Gonzales-Martinez et al Genetic resources in maritime pine (Pinus pinaster Ait.) : are molecular and quantitative measures of genetic variation correlated?</td>
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<td>20:10 - 20:30</td>
<td>A3-9 Katri Karkkainen et al Adaptive variation and genetic differentiation in marginal populations</td>
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**Note:** The schedule includes a mix of presentations and discussions, with specific topics and speakers indicated for each segment.
## TUESDAY December 3rd

**Plenary Session Invited presentations Room S1.4**

**Moderator** Bart Thielges

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<td>Remy Petit</td>
<td>Imprints of colonisation on genetic diversity</td>
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<td>9:05 - 9:40</td>
<td>James Clark</td>
<td>Tree migration capacity and rapid climate change</td>
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<td>9:40 - 10:15</td>
<td>Richard Bradshaw</td>
<td>Forest history and anthropogenic influence on past genetic diversity</td>
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<td>10:15 - 10:45</td>
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<td>10:45 - 11:20</td>
<td>Kermit Ritland</td>
<td>Genetic relatedness in natural populations of forest trees: measurement and experimental results</td>
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<tr>
<td>11:20 - 11:55</td>
<td>Reiner Finkeldey</td>
<td>Genetic implications of silvicultural regimes</td>
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**Parallel Session Room S1.4 Parallel Session Room S1.5**

**Moderators:** Victoria Sork, Martin Lascoux, Paolo Menozzi, Andrew Lowe

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<td>13:30 - 13:50</td>
<td>Ludwig Triest et al Hybridisation in a european willow species complex: information from microsatellites, haplotypes and dominant markers</td>
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<tr>
<td>13:50 - 14:10</td>
<td>Antonio Gonzalez-Rodriguez et al Hybridization between mexican red oaks: morphological and genetic patterns of variation</td>
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<tr>
<td>14:10 - 14:30</td>
<td>Sally Aitken et al. Spatial structure and genetic dynamics of hybrid populations in the Picea sitchensis x glauca introgression zone</td>
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<td>14:30 - 14:50</td>
<td>Xiao-Ru Wang Diploid hybrid speciation of a high mountain pine Pinus densata</td>
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<td>14:50 - 15:10</td>
<td>Caroline Saintagne et al. Genomic hot spots of genetic differentiation between European sympatric oak species</td>
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**Posters Session**

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<td>Giuseppe Vendramin et al. Phylogeography and post-glacial migration history in some broadleaved and conifer forest species as revealed by organelle DNA markers</td>
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<td>Dierk Kownatzki et al. Adaptational characteristics of autochthonous populations-consequences for provenance delineation</td>
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<td>8:50 - 9:10</td>
<td>A4-2</td>
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<td>Ivan Scotti et al. Phylogeography of Norway Spruce in the Alps: combined analysis of nuclear and chloroplast microsatellites reveals genetic structuring and past history of populations</td>
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<td>Sylvain Jeandroz et al. Genetic variation among autochthonous and planted populations of Picea abies from the Vosges mountains in reference to the other French populations assessed by RAPD and mtDNA markers</td>
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<td>Christopher Dick et al. Neotropical differentiation of an ancient rainforest tree, Symphonia globulifera Linnaeus</td>
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<td>Wladyslaw Chalupka et al. Verification of the model of genetic improvement of Scots Pine in Poland</td>
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<td>Claire Williams et al. The Lost Pines: a test of the retreating edge model</td>
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<td>Aristotelis Papageorgiou et al. Loss of alleles due to resistance breeding: the case of Cypress</td>
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<td>Elizabeth Gillet et al. May inclusion of trait differences in phylogeographic analysis alter our views?</td>
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<td>Jacques Louis De Beaulieu et al. Dynamics of forest tree biodiversity: linking genetic, paleogenetic and plant historical approaches (FOSSILVA project)</td>
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<td>Wickneswari Ratnam et al. Effects of selective logging on forest structure and genetic diversity of Shorea curtisii Dyer ex King (Dipterocarpaceae)</td>
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<td>Antoine Kremer et al. Colonisation dynamics and diversity of adaptive traits in European oaks.</td>
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<td>Juan Jose Robledo-Anuncio et al. Pollen movement under alternative silvicultural practices in native populations of Scots Pine (Pinus sylvestris L.) in central Spain</td>
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<td>Jason McLachlan et al. Tree migration capacity under rapid climate change: evidence from the early Holocene</td>
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<td>B2-4</td>
<td>Conservation strategy and management policy must consider both local and rangewide scales: preservation of natural genetic variation in the widespread tree species Spanish Cedar</td>
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<td>Inventory of the genetic diversity in Black Poplar (Populus nigra L.) across Western Europe. Consequences for conservation and nature development</td>
<td>M. Smulders et al</td>
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<td>Potential gene flow between the endangered Black poplar (Populus nigra) and cultivated poplars. A case study along the Meuse of the Dutch-Belgian border</td>
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<td>B3-3</td>
<td>Genetic diversity in natural populations of Black Poplar in Ukraine</td>
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<td>In situ genetic conservation of a naturally restricted and commercially widespread species, Pinus radiata.</td>
<td>Deborah Rogers</td>
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<td>Experiences on ‘ex situ’ conservation of forest trees in Hungary using PCR-markers</td>
<td>Sandor Bordacs</td>
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<td>Effects of domestication on the evolutionary potential of european Chestnut (Castanea sativa Mill.)</td>
<td>Fiorella Villani et al</td>
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<td>B3-7</td>
<td>The domestication of the cultivated cherry trees (Prunus avium) : comparison between genetic diversity of wild and cultivated cherry trees</td>
<td>Muriel Tavaud et al</td>
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<td>B3-8</td>
<td>Peter Zelev et al Genetic diversity in large old growth and in small fragmented Oak stands: implications for management and conservation practice</td>
<td>Kristine Van der Minjsbrugge et al</td>
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<td>Conserving autochthonous genes in Flanders: Creation of forest reproductive material based on inventories and on molecular marker data</td>
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<td>8:30 - 8:50</td>
<td>Jozef Turok Collaborative networking on forest genetic resources in Europe</td>
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<td>8:50 - 9:10</td>
<td>Lennart Ackzell End users needs - view points from a policy maker</td>
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<td>9:10 - 9:45</td>
<td>Thomas Geburek Forest gene conservation in Europe with special reference to widely occurring species</td>
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<td>9:45 - 10:20</td>
<td>Peter Rotach Conservation strategies for rare and disseminated species: Genetic, demographic, biological and practical considerations</td>
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<td><strong>COFFEE, TEA BREAK</strong></td>
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<td>10:50 - 11:25</td>
<td>François Lefèvre Domestication and sustainable management of forest genetic resources</td>
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<td>11:25 - 12:15</td>
<td>Wrap up by session moderators (5 minutes each)</td>
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<td>James Hamrick Maintaining forest tree genetic diversity in the context of changing environmental conditions</td>
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**END OF THE DYGEN CONFERENCE**
ABSTRACTS

INVITED PRESENTATIONS
Hedrick, Phil

Department of Biology, Arizona State University, Tempe, Arizona 85282, USA

* indicates the presenting author(s)

RECENT DEVELOPMENTS IN CONSERVATION GENETICS

Genetics studies in endangered species have become widespread in the past decade, and with new information from various genome projects, new applications and insights are forthcoming. Generally, neutral variants are used for conservation applications, and with highly variable loci and/or many more markers, these approaches should become much more informative. However, conservation genetics is also concerned with detrimental and adaptive variation. Identification and characterization of such variation is more difficult but the ability to predict the extent of detrimental and adaptive variation might become more successful and applied in future conservation. Neutral variants might be used to identify adaptive variants but the overlay of different mutational processes and selective regimes suggests that great caution should be used in making such predictions. Examples of detrimental and adaptive variation in endangered species will be discussed.
MEASURING POLLEN FLOW IN FOREST TREES: A COMPARISON OF ALTERNATIVE APPROACHES

With our increasing human footprint on forest landscapes, genetic fragmentation and its consequences for genetic cohesion are a matter of some concern. We need to be able to assess ‘real time’ gene flow in forest species, but our traditional estimates of gene flow, based on assumptions of drift – migration equilibrium, are inadequate for such disrupted populations. Most recent work has focused on a pair of methodological alternatives, neither of which is dependent on assumptions of evolutionary equilibrium, and we discuss both here. The more direct approach is provided by paternity analysis, the essence of which is to use genetic markers to determine the precise father for several seedlings from each of a few focal mothers, then to measure the maternal-paternal distances, determining the distribution of realized pollination distances. Complications follow from the fact that fathers may be quite distant, and it is impossible to assay all potential fathers. Likelihood methods have been developed that allow us to utilize incomplete information, and we can both estimate the decay rate of short-range pollination probability with distance and gauge the probability of long-distance pollination. Two generation analysis of maternal and seedling genotypes yields an indirect method of assessing the distribution of successful pollination distances. Here, the concentration is placed on the divergence of the pollen pools sampled by different mothers, spaced out across the landscape. The correlation of male gametes within a female, relative to the total sample, can be translated into statements about average pollination distance and the effective number of pollinators. Typically, many (widely-spaced) mothers should be used, with only a few seedlings each. Parentage methods routinely indicate that the decay of short-range pollination probability is sharp but that long-range pollination accounts for a substantial fraction of the total pollination, with many fathers contributing. Conversely, two generation methods indicate that the average pollination distance is generally quite short, and that the effective number of pollinators is often quite small. Two question arise: (1) How can the effective number of male parents be so small if so many males contribute? (2) How can average pollination distance be so short if long-range pollen flow is demonstrable? Very few studies have been mounted that will allow us to compare estimates from both methods directly, but there are some clues in the literature. We compare the inference of the two methods and suggest studies that should help us resolve discrepancies.
HYBRIDIZATION AND ECOLOGICAL DIVERGENCE IN ANNUAL SUNFLOWERS

Entry into new and discrete ecological niches is theoretically difficult because it often requires simultaneous changes at multiple traits. One possible mechanism by which this difficulty might be overcome is transgressive segregation, which refers to the generation of extreme traits in segregating hybrid populations. Transgressive segregation is observed for many traits in artificial hybrids, and some authors have suggested that it might contribute to adaptation in nature. In annual sunflowers, the three wild species found in the most extreme habitats (sand dunes, desert floor, and salt marshes) all happen to be of hybrid origin. Possibly, transgressive segregation contributed to niche colonization. This hypothesis was tested by assessing whether (1) synthetic hybrids exhibited the transgressive phenotypes thought to be necessary for colonization of sand dune, desert floor, and salt marsh habitats; (2) transgressive phenotypes were favored by selection in nature; and (3) QTL combinations contributing to transgressive phenotypes in synthetic hybrids also occurred in the natural hybrid species. Results indicate that extreme traits found in natural hybrid species can be accounted for by transgressive segregation. As expected, transgressive segregation in wild species is largely due to complementary gene action and the transgressive phenotypes and individual QTL are under strong directional selection in hybrid habitats. Finally, transgressive QTL combinations in synthetic hybrids are found in natural hybrid species. Thus, transgressive segregation likely facilitated major ecological transitions in annual sunflowers.
Many trees species are known to have steep latitudinal (or longitudinal) clines with respect to traits related to timing of growth and reproduction. One of the best known clines is for timing of bud set in one year old seedlings of Scots pine. The adaptive nature of this variation has been shown by early transfer experiments between latitudes and altitudes, and by a comparison of presumably neutral markers, which show no evidence of clinal differentiation. We have measured the level of additive genetic variation in Finnish populations. The patterns of clinal variation have also been estimated for the timing and rate of first year height growth. The available predictions on the effects of climate change on the length of growing season are used to evaluate how Scots pine populations could adapt to the changing environmental conditions. Scots pine is a widely distributed species with effective pollen flow, we also discuss the potential of other species with different population structures.
Understanding the forces shaping genetic variation makes it possible to use observed patterns to infer evolutionary processes. Neutral processes allow explicit predictions to be made, such that a comparison across loci permits inferences about mutation, migration and drift. Because migration and drift act evenly on all nuclear loci, we can infer that those loci which show discordant patterns of variation may be experiencing either contrasting mutation rates, or locus-specific natural selection. However, in changing environments, populations are frequently out of equilibrium with patterns of migration and drift. This can induce a high locus-to-locus heterogeneity in patterns of variation, mimicking the action of natural selection. Coalescent modeling indicates that even highly contrasting patterns of variation can be consistent with neutral evolution at all loci. The same reasoning can be applied to quantitative traits, provided that genetic variation in traits is explicitly measured. Generally, traits exhibit greater population divergence than do putatively neutral genetic markers, consistent with locally adaptive selection acting on traits. However, the correlation of marker divergence with trait divergence is not strong enough to be predictive. The individual loci underlying quantitative traits (QTLs) represent the basic adaptive potential for response to future environmental changes, but these are the hardest to study directly in natural populations. Theoretical simulations suggest that such loci will exhibit patterns very similar to neutral marker loci, in spite of strong selection on the traits they code for. This can occur because spatially heterogeneous selection imposes a covariance of allele frequencies across populations, so that traits diverge to a greater extent than the allele frequencies at the corresponding QTLs. This holds out the possibility of using simple genetic markers to draw inferences about the distribution and amount of allelic variation (adaptive potential) at QTLs, but such conjecture remains to be empirical confirmed.
IMPRINTS OF COLONISATION ON GENETIC DIVERSITY

The study of the evolutionary consequences of colonisation is a classic in evolutionary biology (e.g. adaptive radiation following island colonisation) but also and somewhat paradoxically a long-neglected topic in population genetics, a consequence of the domination of ahistorical (equilibrium) approaches in the field. Explicit studies of colonisation and of its consequences on genetic diversity are indeed recent and exemplify the better integration of space and time in empirical and theoretical population genetics. In this talk, we will particularly address the maintenance of genetic diversity for both neutral and adaptive genes during the colonisation process, by emphasising situations when diversity is better preserved than might be expected in simple models. We argue that this question is of special interest, because it may account for the evolutionary success of the populations and species experiencing repeated environmental challenges leading to range shifts. The factors that can mitigate (or exacerbate) diversity loss include (but are not limited to) the following: (1) Demographic factors: frequency and size of founding events, origin of colonists involved, shape of colonisation routes, admixture of differentiated populations. (2) Gene flow: mating system shifts caused by differences in density or by edge effects. (3) Selection: balancing selection, selection for increased dispersal ability, for increased gene flow to resist or avoid inbreeding depression by rescuing newly established populations; release of selective forces operating in dense populations or on the contrary occurrence of selective sweeps under new environmental conditions. (4) Mutation: increased mutation rate under stress conditions. (5) The interaction between any of these factors. We will use the orbitally-induced Quaternary migrations of forest tree species as a paradigm to illustrate the genetic consequences of postglacial (re)colonization, by reviewing empirical data (based on nuclear and organelle markers) and theoretical models in the field and by discussing the factors that were identified in these studies.
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TREE MIGRATION CAPACITY AND RAPID CLIMATE CHANGE

The ranges of many North American trees will have to expand at rates of 100 to 1000 m yr⁻¹ to track predicted climatic changes of this century. Interpretations of fossil pollen data suggest that such rates were typical for tree populations responding to postglacial warming and provide optimism that rapid migrations could occur again. These interpretations are consistent with deterministic models of population spread parameterized with tree fecundity and seed dispersal data, which imply accelerating expansion that achieves high velocities in short amounts of time. To address several unrealistic aspects of the models of spread and of the statistical models used to parameterize them we developed alternative approaches that raise doubts about the rapid rates interpreted from paleo data and predicted by deterministic models. Parameter estimates based on a hierarchical Bayes framework indicate that previous parameter estimates of fecundity are unrealistically high and those of dispersal are unrealistically long. The bias in previous models results from inappropriate treatment of stochasticity (Clark, LaDeau, Ibanez, in prep.). Stochastic models of spread that are based on these estimates predict rates at least an order of magnitude lower for the same species. Although models do not rule out extraordinarily rare, long distance dispersal events, the disparities between paleodata and model results are dramatic and consistent across a wide range of species (Clark, Lewis, HilleRisLambers, McLachlan, Ecology, 2002). We reevaluate paleo interpretations of population spread using molecular evidence that provides insight into how postglacial spread was accomplished. The predictions of slow spread led us to hypothesize a distribution of glacial tree populations that would be difficult to identify from fossil data and that might explain why past migrations were not rapid. We used chloroplast DNA surveys to show that the geography of post-glacial range expansion in two eastern North American tree species differed substantially from pollen based reconstructions and from patterns emerging from European molecular studies (McLachlan, Clark, Manos, in prep). Our molecular evidence suggests that American beech (Fagus grandifolia) and red maple (Acer rubrum) persisted as low-density populations close to the Laurentide Ice Sheet, at higher latitudes than interpreted from pollen. Because populations were much further north than previously thought, postglacial migration rates were slower than those estimated from fossil pollen. Our estimated rates of <100 m yr⁻¹ are consistent with stochastic model predictions. This new evidence suggests that past migration rates were substantially slower than the rates that will be needed to track 21st Century warming. The combination of empirical evidence and model studies indicates that tree species are at risk of extinction as climates warm during the next century.
FOREST HISTORY AND ANTHROPOGENIC INFLUENCE ON PAST GENETIC DIVERSITY.

Present population structure and patterns of genetic diversity in forest trees have been influenced by past anthropogenic activities and the major aim of this paper is assess the scale and timing of this influence within Europe. Very little 'virgin' forest (defined as areas that have been continually forested since conditions became suitable for tree growth) remains in Europe. Recent estimates are well below 1% of forest area in all regions except for Scandinavia, former Soviet Union and parts of the Alps and Balkans. Yet many contemporary European forests have developed by natural regeneration from local stock, so despite a massive anthropogenic impact, one can predict a survival of the 'natural' patterns of genetic diversity despite a general reduction in total diversity. Denmark, for example, contains no virgin forest, but only an estimated 5% of all Fagus trees are of foreign provenance. Systematic interference with forests was closely associated with the spread of agriculture from south-east to north-west Europe during the last 10 000 years. It has been suggested that the spread of trees of early economic importance (e.g. Corylus, Fagus, Quercus) was linked to local agricultural development. However closer analyses of spreading dynamics and comparisons with comparable spreading in the USA does not favour this hypothesis. Indeed modelling the present and past range limits of forest trees has suggested that climate change is the major driving force for range changes, implying that genetic composition is primarily governed by natural processes. By the Roman period, there are documented cases of introduction of e.g. Aesculus hippocastanum and Castanea sativa to Britain. Countries with colonial histories have been importing undisputedly 'exotic' species for several centuries. Occasionally there is doubt over exotic status owing to lack of documentation or possible prehistoric introduction, for example Arbutus unedo in western Ireland. The translocation of native species with the aim of improving production potential is a relatively recent practice often documented in national histories of forestry. Anthropogenic influence on European forests has been considerable and European temperate deciduous forests together with the broad-leaved evergreen Mediterranean forests are among the most modified ecosystems in the world. Systematic alteration dates back to the spread of agriculture. Much genetic diversity must have been lost and the relative abundances of forest species has been significantly altered. However, long-distance translocation of trees is a relatively recent management practice confined to documented plantations. Thus the patterns of genetic diversity observed in naturally regenerated forests primarily arise from natural processes.
GENETIC RELATEDNESS IN NATURAL POPULATIONS OF FOREST TREES: MEASUREMENT AND EXPERIMENTAL RESULTS

Developments in molecular techniques are continuing to make genetic markers more effective for measurement of relatedness and population structure, and are widening the scope of their application in forest population genetics. While most forest trees show little population structure, recent dispersal within local neighborhoods generates significant local structure. And while individual estimates of relatedness can show high statistical variance, inferences involving many individual estimates can be significant. In this talk, the basic framework for estimating genetic relationship is first given, including certain multistage designs to maximize statistical power. Then, applications of pairwise estimates are discussed. These include the estimation of heritability “in the field”, the “spatial covariance of relatedness”, and the “inbreeding genetic load”, using forest trees such as Tsuga, Abies, and Quercus. I will also attempt to suggest some directions for future research in this area.
GENETIC IMPLICATIONS OF SILVICULTURAL REGIMES

Human impacts on genetic structures of forest tree populations are manifold. Changes of environmental conditions and forest stand structures brought along by silvicultural practices concerning regeneration, thinning, and harvesting operations have a strong influence on evolutionary forces like drift, mating system, gene flow and selection. The observation of long-term effects of silvicultural regimes on genetic structures and adaptive potentials is hampered by long rotation cycles of most forest trees. Artificial regeneration is the most obvious silvicultural practice resulting in possibly drastic changes of genetic structures not only in planted forests, but, in later generations, due to gene flow also in neighbouring forests. However, natural regeneration is no safeguard against unintentional changes of genetic structures by forest managers. Losses of genetic variation and adaptive potentials may be expected if reproduction-effective population sizes are seriously reduced or, mainly in case of rare and scattered species, if the gene flow through pollen is severely restricted by low population densities. Harvesting and thinning operations may have serious impacts on adaptive and economically important traits due to selection in favour of particular phenotypes. Target diameter felling serves as an example to describe possible consequences for genetic structures on subsequent generations in more detail.
COLLABORATIVE NETWORKING ON FOREST GENETIC RESOURCES IN EUROPE

The European Forest Genetic Resources Programme (EUFORGEN) was established in 1995 as the implementation mechanism of Strasbourg Resolution S2 («Conservation of Forest Genetic Resources») adopted at the first Ministerial Conference on the Protection of Forests in Europe. The overall goal of EUFORGEN is to ensure the effective conservation and the sustainable use of forest genetic resources (FGR). The Programme operates through five Networks for target groups of species. Network members from participating countries carry out an agreed workplan, with their own resources, as inputs in kind. EUFORGEN is financed by its participating countries and is coordinated by the International Plant Genetic Resources Institute in close collaboration with the UN Food and Agriculture Organization. EUFORGEN is overseen by a Steering Committee of National Coordinators nominated by the participating countries. The collaborative activities of the Networks include regular exchange of data and information, development of technical guidelines and common standards, preparation of joint project proposals, exchange of genetic materials, literature overviews and public awareness. A large number of practical outputs have been provided to date. These will be demonstrated during the Conference through individual poster presentations of each Network. The main objective during current phase II (2000-2004) is to complete a set of technical guidelines for forest officers and agencies responsible for FGR at national level. The EUFORGEN Networks have provided a very active and efficient forum for the exchange of experience and information. Thirty-two European countries have participated in the Programme. During this period, a spirit of cross-border collaboration has been established. The strong endorsement by the participating countries of the work conducted within the framework of EUFORGEN confirms the importance of FGR for future sustainable forest management in Europe, timely recognized by adopting Resolution S2 at the First Ministerial Conference in Strasbourg. New knowledge gained from research projects on forest genetic diversity in Europe (mainly funded by the European Union during recent years), together with new biotechnological tools, has become available to support the practical conservation of FGR. With the established, frequent contacts in the European forest community, the EUFORGEN Networks provide unique opportunities for facilitating linkages and enhancing collaboration in pan-European research. All the Networks have been involved in the planning, developing and submitting research project proposals. Besides the support for complementary research activities and the joint project development, the regular Network meetings – a major tool in the Network’s operation – have provided a platform to disseminate and promote the implementation of the results once a research project is completed.
END USERS NEEDS - VIEW POINTS FROM A POLICY MAKER

In the best of worlds political decisions are based on scientific results. The possibility to show such results is often influential in the debate. The policy makers’ mandate in funding and legislation and their need of scientific results create an important interdependency situation between policy and science. The policy maker is under heavy information pressure from many sources. It is a daily duty to analyse and balance different wishes. This political process includes risk assessments, media trends, actuality of the topic, etc., all part of transparent western democracies. Science has to be a visible part of the information to non-specialists. An awareness of the policy maker’s world is a prerequisite to provide the results in the right format. There are two fundamental reasons for researchers to ask for funds, which also form the basis for most political decisions; threats or prosperous possibilities. In the field of forest genetic research this applies to e.g. gene conservation, breeding, GMO, tracing origin of wood or forest seeds, provenance adaptability, clonal forestry, and exotic species. All of these are tasks for policy and legislation and they all include aspects of threats and possibilities. From a policy makers view point, as an end user of scientific results, the most important thing is to establish bridges between research and policy. Promising initiatives are e.g. - the EU funded Oak flow project which includes both science and policy/implementation levels, - popular publication series of results e.g. Skogsfakta, in Swedish from the Faculty of Forestry, - the set-up of multi sectorial advisory groups and - the initiative of this very conference and invitation leading to this presentation. Across these bridges the information flows should steer both policy and science in a wise way without reducing the scientific freedom of wild thinking and the policy maker’s obligation to take into account other sectors or the demands of society.
FOREST GENE CONSERVATION IN EUROPE WITH SPECIAL REFERENCE TO WIDELY OCCURRING SPECIES

The overall goal of forest gene conservation is often defined as the preservation of functioning genetic mechanisms that confer on tree populations the ability to adapt to environmental changes and to maintain genetic diversity as the «building bricks» for breeding activities. But how can this general definition be applied to particular situations, for instance to widespread forest tree species? It is clear that as it stands, this objective is not epistemologically applicable, that is, we simply neither can set a specific time frame within the above-mentioned objective is to be fulfilled nor can we precisely say when genetic mechanisms are preserved. The objective and its fulfilment must remain vague. This is also due to a difficult assessment of potential threats which exemplarily will be demonstrated in this paper referring to local deforestation (fragmentation) through human activities or environmental pollution and translocation of forest reproductive material. However, threats will be often unknown. Consequently, data compiled how forest gene conservation are implemented in Europe mainly through legally and non-legally binding instruments, genetic reserves, seed stands, and seed orchards are of limited value and it is difficult comparing efficiencies reaching the conservation goal in different regions. Furthermore, experimental data on processes that may modify and shape adaptive diversity are very scarce and in most cases only educated guesses are possible when selecting conservation populations, estimating minimum population sizes, and working out technical guidelines. At the same time the rationale of certain specific action - explicitly the declaration of gene reserves for widely occurring European tree species - will be questioned. Instead, a holistic and intersectoral ecosystem approach that integrates the conservation and sustainable use of genetic diversity, also taking account of social, cultural and economic considerations is advocated.
Rarity is not a straightforward notion. Rarity exists in different forms, each with its own set of causes and consequences regarding vulnerability, conservation needs and conservation strategies. While most endangered species listed in the red list are rare in one or the other way, the opposite is not true. Rare species do not necessarily have higher conservation needs than wide-spread species. Consequently, endangerment and conservation needs for rare species need to be assessed individually for each species and specific situation. Since there is no such thing as a rare species, adapted species specific conservation strategies are required. Development of in situ conservation strategies for rare species need to be based on a thorough analysis of the population structure and form of rarity, the specific causes or processes which led to the current situation of rarity, the past demographic development and future prospects and information on the special life-history traits, the reproductive biology and the genetic system of the species in question. Although a lot of this information is still missing for most of our rare species, educated guesses allow to develop species specific and adapted conservation strategies as is demonstrated by two examples from Switzerland. In addition to the development of clear and realistic strategies for each species and situation, attention needs to be paid to the practical implementation. Because rare and disseminated species need large areas and require active silvicultural management, conservation and promotion activities will be most effective and efficient if they are integrated into ordinary forestry. This, however, requires certain instruments as well as trained and motivated partners in the forest service.
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DOMESTICATION AND SUSTAINABLE MANAGEMENT OF FOREST GENETIC RESOURCES.

Temperate and Mediterranean forest ecosystems in Europe suffer various degrees of human impact. Moreover, the multiple forest uses concern both cultivated and non cultivated stands, i.e. devoted or not to production. Finally, cultivated and non cultivated stands often share the same territory. Therefore it seems necessary to consider the forest genetic resources as a whole, including both cultivated and wild populations with possible interactions. I propose to extend the concept of domestication to all the genetic modifications of spontaneous populations which could result from human activities, including: (i) direct selection through breeding and sylviculture, (ii) transfer and spread of selected material, (iii) direct control of demography and reproduction processes, (iv) modification of the evolutionary processes through the alteration of the environment. In order to investigate the main impacts of human activities on the adaptability of forest genetic resources, I briefly review the knowledge on domestication processes in plants and on interaction between cultivated and non cultivated compartment. Recent results allow us to make predictions on the evolution of genetic diversity and give first recommendations to the managers. However, genetic diversity is only one component of the adaptability of the ecosystem and further research is deeply needed to understand the evolution of adaptation and plasticity of populations: these fields of research will require integrative and multidisciplinary approaches.
MAINTAINING FOREST TREE GENETIC DIVERSITY IN THE CONTEXT OF CHANGING ENVIRONMENTAL CONDITIONS

Forest tree species have many characteristics that may uniquely situate them to withstand the effects of human induced environmental changes. Paleoecological evidence has demonstrated that the geographic ranges of forest tree species have expanded and contracted several times since the last glacial epoch in response to environmental changes. These range fluctuations have been accomplished without any apparent loss of genetic diversity. A possible explanation that may distinguish forest trees from many herbaceous species is that much of the genetic variation within tree species is found within rather than among their populations. This appears to be true (but perhaps to a lesser extent) for traits undergoing selection as well as for neutral genetic markers. Thus, the extinction of a relatively large proportion of a tree species' populations would result in relatively little loss of genetic diversity. Furthermore, phylogeographic studies indicate that gene lineages originating from separate glacial refugia have maintained sufficient genetic variation to allow them to adapt to novel habitats as the species expanded their ranges following glacial retreat. It is sometimes overlooked that habitat heterogeneity (elevation, slope, aspect, moisture etc.) in the refugia may have preserved adaptive genetic variation that, when recombined and exposed to selection in newly colonized habitats, has given rise to the local adaptation that we see in present-day tree populations. The maintenance of genetic diversity in the face of extensive habitat fragmentation is also a special concern for modern landscapes. Forest trees may, however, be buffered from the adverse effects of such fragmented landscapes. First, the longevity of individual trees may retard population extinction and allow individuals and populations to survive through the period of fragmentation until habitat recovery occurs. Second, considerable evidence is mounting that most forest tree fragments experience levels of pollen flow high enough to counteract the effects of genetic drift. This observation appears to be true of animal as well as wind-pollinated tree species. In conclusion the combination of individual longevity, high intra-population genetic diversity and the potential for high rates of pollen flow should make forest trees especially resistant to the loss of genetic diversity while undergoing changing environmental conditions.
ABSTRACTS

VOLUNTARY PRESENTATIONS
CONTEMPORARY POLLEN MOVEMENT WITHIN AND AMONG WIND- AND INSECT- POLLINATED TREE SPECIES.

Forest management and landscape changes are modifying the spatial structure of tree populations. Some populations are experiencing reductions in conspecific density, others are experiencing changes in surrounding forest structure, and yet others have increasing isolation among individual trees. A critical question is whether these changes in landscape context are affecting contemporary patterns of gene flow and the resulting mating neighborhood of trees.

We have developed a method of measuring "real time" pollen-mediated gene movement that uses the structure of the pollen pool sampled by maternal plants scattered across a landscape to estimate the effective number of pollen donors, effective neighborhood size, and the average distance of effective pollen movement. Using this TwoGener model, we look at three wind-pollinated tree species. In the first two cases, the species share the same Missouri Ozark landscape but differ in conspecific density. Quercus alba has an effective pollination neighborhood with circle of radius ~20 m and ~ 8 effective pollen donors, while the low-density population of Pinus echinata has a similar neighborhood area but only about 2 pollen donors/maternal tree. In Pinus echinata, we also compared results across three management treatments -- selective cutting, clear-cutting, and uncut second growth forest -- and found that both neighborhood area and the effective number of pollen donors were highest among trees left in the clear cut treatment, suggesting the well-developed forest canopy restricts pollen movement. The third wind-pollinated species, Quercus lobata, which was studied in oak-savanna landscape, exhibited greater pollen dispersal than the first two species, but the estimate of pollen donor number was only about 4 individuals in one year and a bit higher in the next. We present the results from two insect-pollinated species: Cornus florida in Missouri Ozark forest and Albizia julibrissin (Irwin et al, in press) for populations in Georgia. For Cornus, we estimated 8-11 effective pollen donors; for Albizia, only 2-4 donors. These results indicate that pollen donor neighborhoods for insect pollinated species can show the same range as wind-pollinated species, but too few studies are available to allow wider generalization at this time. Given the assumptions of the TwoGener model, we need to think more about the parameters of the model that can be estimated for insect pollinated-species. The conclusions of this talk will address both the biological inferences of current studies of contemporary pollen movement and the application of the TwoGener to future investigations.
ESTIMATING POLLEN FLOW IN TREE SPECIES WITH VARIOUS LIFE-HISTORY TRAITS

Gene flow through pollen is a major process that shapes genetic diversity in plants. The aim of this study was to estimate the mean distance of pollen dispersal and the dispersal curve for several tree species with contrasting life-history traits: social species or scattered species, monoecious or dioecious species, species from temperate and tropical forests. For this purpose, we performed for each species a paternity analysis on seeds sampled in a given population on several mother trees, using codominant molecular markers. In parallel we studied these species with the TwoGener method, which is based on the estimation of differentiation among the pollen pool fertilizing the sampled females. The advantage of this method is that it does not require the exhaustive sampling of the males of the population to estimate pollen flow. Both methods yield concordant results. (i) The estimated pollen dispersal curves are fat-tailed, yielding on one hand an excess of crossing between close individuals but on the other hand a non-negligible proportion of long-distance dispersal. (ii) Male reproductive success was highly variable among individuals, which lead to a lower effective density of reproducing males compared with the observed density. (iii) The dispersal curves obtained are consistent with the species’ life-history traits.
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BIPARENTALLY, MATERNALLY, AND PATERNALLY INHERITED MOLECULAR MARKERS ELUCIDATE POPULATION PROCESSES ON A SMALL SCALE WITHIN A NORWAY SPRUCE STAND

Gene flow studies in plants have been limited to indirect estimates on sex-specific contributions mainly due to a lack of paternal markers or because of the relatively low degree of genetic variation in the haploid organelle genomes. In many gymnosperm species, however, the cytoplasmic genomes are differentially inherited, which provides an excellent opportunity for the study of population genetic structures resulting from past and present dynamic processes. We analyzed nuclear (n), chloroplast (cp) DNA, and mitochondrial (mt) DNA variation in Norway spruce (Picea abies) in a mixed stand located in northern Italy. In P. abies, mtDNA is maternally transmitted, whereas cpDNA is paternally transmitted, and polymorphic molecular markers are available for both these genomes as well as for nDNA. We aimed to resolve the genetic relationships, with respect to the spatial arrangement, among all individuals in order to infer maternal and paternal contributions to gene flow within the stand. Our preliminary results show that the genetic variation detected by the three marker types decreased from nuclear simple sequence repeats (SSRs) to cpSSRs and to mtDNA tandem repeats. Nuclear loci indicate that observed heterozygosity is higher in mature rather than juvenile trees, but that expected heterozygosity and expected number of alleles are not statistically different in the two age classes. In multivariate spatial autocorrelation analysis, nDNA revealed no spatial structure whatsoever, whereas cpDNA indicated a positive autocorrelation for the juvenile trees. In mtDNA, both juveniles and putatively reproductive trees indicated a non-random genetic structure, but at shorter distances than in cpDNA. This structure in mtDNA was even stronger when the two life stages were combined. The differences found between the two organellar genomes may be interpreted as the result of their differential dispersal modes. CpDNA is transferred not only by the wind-borne pollen, but also by seed within the embryo cells. On the other hand, mtDNA is only dispersed via seed, which presumably has a lower dispersal capability than does pollen. On a within-population scale, our findings confirm the predictions of population genetics theory, according to which differentiation should be higher for maternally inherited markers, and reduced for paternally and biparentally inherited markers. The combined analysis of all three marker types should allow us to reconstruct past population processes in great detail, and to better understand family structure and the dynamics of genetic diversity in spruce stands.
How habitat fragmentation may affect the population dynamics of forest tree species is poorly documented. Theoretical consequences of habitat fragmentation are straightforward extensions of population genetics theory but empirical studies conducted so far suggest that gene flow processes respond to habitat fragmentation in more complex and varied ways than first expected. In Southern Scotland, human-mediated aforestation for pasture and subsequent continuous herd grazing has dramatically altered the landscape. Native woodland cover is very limited, with only one percent of native woodlands remaining, most of which is highly fragmented and has been so for several centuries. This situation provides a unique opportunity to assess how extended periods of habitat fragmentation modifies gene flow patterns in forest ecosystems. We intensively sampled a 4km wide valley for Sorbus aucuparia and Fraxinus excelsior forest remnants. We used microsatellite markers to infer contemporary pollen-mediated gene flow by paternity analysis performed on seed collected from mother trees and seed-mediated gene flow by parentage analysis performed on newly established seedlings. Comparison of these estimates with patch size and degree of spatial isolation is discussed. Finally, we present how results are to be used in a native woodland restoration programme currently being implemented.
CONSEQUENCES OF RAINFOREST FRAGMENTATION: A COMPARATIVE GENETIC STUDY ON RARE AND COMMON ELAEOCARPUS SPECIES.

Much of the original rainforests of northern New South Wales (Australia) have been cleared in the last 150 years. Early 'opening-up' occurred mainly because of the demand for red Cedar (Toona ciliata), with subsequent clearing for cropping and dairy farming. A large area of rich volcanic soils known as the 'Big Scrub' was originally covered by lowland subtropical rainforest but is now reduced to less than 1% of its original extent. The Big Scrub and surrounding areas have an important evolutionary role as they provide a bridge between northern and southern rainforests. The Big Scrub remnants represent a complex matrix of differently sized and isolated fragments. As this area is surrounded by comparatively undisturbed rainforest, it represents an ideal study case for in-depth investigations on how fragmentation affects population dynamics. A microsatellite-based study is currently investigating how population size and isolation affect gene flow in three Elaeocarpus (Elaeocarpaceae) species. Microsatellites were developed for a common species, E. grandis, and were used across two rare species, E. williamsianus and E. spp Minyon. Genetic data was obtained for all known individuals of the two rare species producing some surprising results, such as the presence and extent of clonality in E. williamsianus. Close to 500 E. grandis trees, representing most of the NSW populations, were also analysed. A selection of undisturbed and fragmented populations is currently being investigated in greater detail. Seeds have been collected and are contributing direct measures of gene flow within different population types. This study provides new insights into the contrasting effects that fragmentation has on rare and common species.
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ESTIMATING POLLEN VERSUS SEED DISPERSAL FROM SPATIAL GENETIC STRUCTURE IN THE COMMON ASH

Gene flow in the form of pollen and seed dispersal is a key determinant of spatial genetic structure in natural plant populations. When overall gene flow is restricted, genetic relatedness among pairs of individuals decreases with increasing geographic distance in a way predicted by isolation by distance models. In many forest tree species, spatial genetic structure has been investigated using molecular markers and the tools of spatial autocorrelation analysis. The observed spatial genetic structure can be used to estimate local levels of gene flow with methods that have recently been developed for this purpose. However, the influence of the relative contributions of pollen and seed dispersal on those inference methods is not fully understood yet. Common ash (Fraxinus excelsior L.) is a wind-pollinated and wind-dispersed tree species that widely occurs in mixed deciduous forests nearly all over Europe. We have analysed spatial genetic structure in a common ash population from southern Romania using five nuclear microsatellite loci. The influence of the relative contributions of pollen and seed dispersal on spatial genetic structure was investigated through intensive computer simulations on a theoretical population matching as closely as possible features of the population under study. The objective was to find out whether inference of pollen versus seed dispersal could be drawn from the observed spatial genetic structure in this natural common ash population.
Limited seed dispersal in a partially scattered tree species, *Fraxinus excelsior* L., as revealed by parentage analysis using microsatellites

Common ash, *Fraxinus excelsior* L., is a partially scattered temperate forest tree with a colonizing behaviour, a discontinuous spatial distribution and a complex mating system (continuum from pure male individuals to pure female individuals with all kinds of hermaphrodites in between). It is a wind-pollinated species and the fruits are also wind-dispersed. Using five polymorphic microsatellite loci developed on this species, we conducted a parentage study in a managed stand located in France and made of 597 adult trees. A hundred natural seedlings belonging to 4 different zones were sampled and located with a GPS. First, we found that the mean dispersal distance for seeds in this population is about 140 meters. This value is significantly lower than the one expected at random, which means that seed dispersal within the studied stand is limited. Moreover, the amount of gene flow from outside the stand is high (45.5 %). Following the usual hypothesis that the maternal parent of a seedling is the closest of the two parents, we can conclude that the main part of this gene flow from outside the plot is due to dispersal by pollen. This gene flow from outside the stand can not be accurately quantified in terms of dispersal distance as the parental individuals located outside the plot are not identified. Nevertheless the difference between pollen and seed dispersal can be assessed using the information given by the seedlings for which two parents are found inside the stand. For those seedlings, we measured the ratio ‘distance between the two parents/minimal distance between one parent and the seedling’ that represents the ratio of pollen dispersal distance on seed dispersal distance. In our common ash population, this ratio reaches 8.5 and is significantly higher than that expected at random. This result reveals an asymmetry of dispersal in common ash, pollen migrating further than seeds. Finally, the observed directions of seed dispersal seem to follow the linear structure of the studied population, that-is-to say preferentially along the two small valleys (oriented South-West/North-East and South-East/North-West). Seed dispersal in this stand seems in fact to follow the landscape topography. In conclusion, the patterns and levels of seed dispersal observed in common ash will be discussed in the light of common ash biological characteristics and by comparison with what is found in other tree species.
GENETIC STRUCTURE AT DIFFERENT SPATIAL SCALES IN AN ENDANGERED, FIRE-DEPENDENT PLANT.

The genetic consequences of a fragmented distribution are expected to be most pronounced in species that form small subpopulations, are fully self-compatible and have limited seed dispersal. The rare, Australian shrub species, Grevillea macleayana (Proteaceae), has these characteristics, but its long-lived seed banks, mobile pollinators and long adult life span possibly attenuate their effects. Using microsatellites, we found that Grevillea macleayana appears to have relatively low within-population diversity, significant population differentiation and moderate genetic structure showing isolation by distance, consistent with historically low gene flow. The frequency distribution of allele sizes suggest that this geographic differentiation is being driven by mutation at some loci. Preliminary results from the sequencing of more slowly evolving chloroplast genes are consistent with the broad scale patterns observed from microsatellites. We found a lack mutation-drift equilibrium at microsatellite loci in some populations that is indicative of population bottlenecks. Combined with evidence for large spatiotemporal variation of selfing rates, this suggests that fluctuating population sizes characterise the demography in this species, promoting genetic drift. Interpreting fine scale or intra population genetic structure in G. macleayana is confounded by the imposition in recent times of anthropogenic disturbances with potentially contrasting genetic consequences: 1. the unusual foraging behaviour of exotic honeybees and 2. widespread disturbance and dispersal of the soil-stored seedbank by road building and quarrying. To test for evidence of fine-scale genetic structure within G. macleayana populations and to test the prediction that such structure might be masked by disturbance of the seed bank we tested for spatial autocorrelation of genotypes at two sites in undisturbed habitat and compared their genetic structure with two sites that had clearly been strongly affected by road building. High selfing levels inferred from genotypes at all four sites implies that pollen dispersal has been limited. Consistent with this we observed substantial spatial clustering of genes at 10m or less in the two undisturbed populations and argue that this reflects the predicted effects of both high selfing levels and limited seed dispersal. In contrast, at the two sites disturbed by road building, spatial autocorrelation was absent or weak. This is consistent with there having been mixing of the seed bank, increasing seed dispersal and counteracting the effects of elevated selfing due to honeybees. It appears that in the past, the potential for spatiotemporal dispersal of genes via long-lived seedbanks and highly mobile pollinators has not prevented the accumulation of genetic structure at local and regional scales in G. macleayana.
LOCAL POLLEN DISPERAL AND DISTANT GENE FLOW IN NORWAY SPRUCE (PICEA ABIES [L.] KARST.)

Pollen gene flow is one of the most important factors influencing genetic structure of wind pollinated forest trees. We investigated mating system, pollen dispersal patterns and the level of pollen immigration in a clonal archive of Norway spruce (Picea abies [L.] Karst.). The plantation consisted of 114 clones originating from different (often distant) parts of Poland. We sampled 100 seeds in upper and lower crown portions of two ramets, each of five clones (totally 2000 seeds) and the parameters of reproductive system were inferred based on allozyme markers and neighborhood model approach. We found that the level of self-fertilization was generally low ($s = 0.059$), although statistically significant. The immigration from outside of the neighborhoods (20 m radius) was considerable accounting for more than 83% of successful fertilizations. Despite the high level of distant gene flow, pollen dispersal within neighborhoods was severely restricted to neighboring trees with a mean effective pollen dispersal distance = 6.8 m (mean physical distance between mothers and potential fathers = 13.1 m). In addition, mating success of individual males within neighborhoods was moderately influenced by their fecundity and direction of their location relative to mother trees. The mean effective number of outcross males within neighborhoods was low (Nep = 6.71) which is about 7.5% of the mean number of potential males (90). The mating parameters were similar in upper and lower crown levels. The implications of reproductive patterns of clonal archives for ex-situ conservation programs is briefly discussed.
HYBRIDIZATION IN A EUROPEAN WILLOW SPECIES COMPLEX: INFORMATION FROM MICROSATELLITES, HAPLOTYPES AND DOMINANT MARKERS

The main objective of the current EU-research project is to use molecular tools as screening methods to determine the genetic identity, structure and dynamics of introgressed populations of riparian willows (DYNAMO= The DYnamic NAture of introgressive hybridization in natural and introduced polyploid plants from agricultural and riparian landscapes: an evaluation of MOlecular tools in willows). To achieve these objectives, controlled crosses as well as plant materials from different European habitats are used. On the progeny of controlled crosses, willow nuclear SSR loci were screened for variability and clarity of amplification in the Salix alba-S. fragilis complex. The results indicated two clear groupings: one containing individuals of S. fragilis with some hybrid plants and the other containing individuals of S. alba and another set of remaining hybrids. Species-specific alleles have been identified at 8 loci. To increase throughput of samples, a fluorescently-based detection system and multiplexing was developed. Inheritance of SSR loci was investigated in controlled crosses between S. alba and S. fragilis. Results indicate that the complex is of allotetraploid origin. The nuclear SSR loci are also useful in detecting clonality among collections and samples from natural and semi-natural habitats. Both willow species Salix alba and Salix fragilis often are dominating trees in riparian vegetations and seepage zones. Natural reproduction may occur through seedling establishment or clonal spread of vegetative propagules. A working hypothesis is that clonal reproduction is more important in upstream stretches, whereas regeneration through seeds could be more important in downstream floodplains. Therefore, the project concentrates on estimating the clonality versus sexual reproduction in populations along river banks; on revealing hybrid identity at dynamic zones of interaction between upstream and downstream ecotypes and genotypes; and on estimating the extent of introgression in natural populations along major European rivers. More than 1000 willow trees were sampled for DNA analysis along major European rivers (Rhine, Danube, Rhône, Taag, Ebro, Evros, Axios, Arno, Adige, Schelde, Meuse, Mosel,...). Different stretches of rivers might reveal different population structures with regard to their dynamics and origin: upstream parts in the mountains, downstream parts in the floodplains, natural riparian zones, secondary populations along abandoned gravel-pits and sand-pits. The use of co-dominant markers is very promising to describe patterns of hybridization at a local scale, while both the dominant markers and haplotypic markers are promising to describe patterns at a larger regional scale. Chloroplast microsatellite variation was detected in four out of the six markers analysed and revealed "species-specific" haplotypes. Despite the low level of cpSSR length variants found in a first set of analysed populations, confirmed by sequencing as differences in number of repeats, a high level of genetic differentiation among populations, geographically structured, was observed. The dominant markers reveal clones and a skewed hybridisation pattern between upstream and downstream parts of particular river catchment areas such as the Rhine. Information of genetic structuring is helpful in understanding the importance of natural functioning of floodplains on hybridization events in riparian willows.
HYBRIDIZATION BETWEEN MEXICAN RED OAKS: MORPHOLOGICAL AND GENETIC PATTERNS OF VARIATION.

Quercus laurina Humboldt et Bonpland and Q. affinis Scheidweiler are two closely related and morphologically similar mexican red oaks. Q. laurina is mainly distributed along elevations of the Sierra Madre del Sur in southeastern Mexico, and Q. affinis along the Sierra Madre Oriental in eastern Mexico. The two species overlap in distribution in the east of the transversal neovolcanic belt. Within this area of overlap, there are many localities where the two taxa are sympatric and the taxonomic assignment of individuals becomes problematic. In contrast, individuals from populations outside of the area of overlap can be unambiguously determined. This pattern of morphological geographic variation may be due, at least in part, to frequent hybridization between the two oak species. This possibility was analyzed using a set of RAPD and morphological markers. Ten typical individuals from allopatric populations from each species were characterized with 131 10-bp primers, which produced 711 markers. From these, eight primers provided 11 markers that appeared to be almost specific to one species or the other. More than 500 hundred trees from 15 populations located within the area of overlap, and two allopatric populations from each species were later characterized using these eight primers. Additionally, nine morphological characters were measured in ten randomly chosen leaves from each individual. Calculation of hybrid index scores from RAPD markers confirmed that hybrids belonging to several genotypic classes are present in most localities where the two species are sympatric. A significant association between RAPD and morphological variation was detected, but only a small amount of the wide range of morphological variation observed can be explained by genotypic variation.
SPATIAL STRUCTURE AND GENETIC DYNAMICS OF HYBRID POPULATIONS IN THE PICEA SITCHENSIS X GLAUCa INTROGRESSION ZONE

Genetic analysis of the introgression zone between Sitka spruce (Picea sitchensis) and white spruce (Picea glauca) in northwestern BC was conducted to determine the genetic composition and evolutionary dynamics of spruce populations within the introgression zone, and to relate genetic composition to geographic and climatic variables. Seed samples were collected from pure Sitka spruce and white spruce populations and from sixty putative hybrid parent trees from sixteen locations across the introgression zone along the Nass and Skeena River drainage basins in northwestern British Columbia, Canada. DNA was extracted from needles of 2-year-old seedlings. Nuclear DNA Sequence-Tagged-Site (STS) markers were used to develop a hybrid index, which reflects the relative contribution of Sitka spruce and white spruce genomes to hybrid populations. Hybrid indices were estimated using Elston's least squares method by performing a multiple regression of allele frequencies of each putative hybrid family on the corresponding allele frequencies of the two parental species. Significant differences in hybrid index were observed among sampling locations and these generally followed a clinal distribution from maritime to more continental climates. Mean hybrid indices of populations decreased from the coast to the interior within the introgression zone in spite of substantial among-family variation at individual locations. The geographic distribution of hybrid indices is complex due to the considerable topographic and associated climatic variation found in the zone of introgression. Physical distance to the nearest marine inlet and longitude both accounted for a significant proportion of the variation in hybrid index. Patterns of variation for hybrid index coincided with those obtained for growth rate, phenology and cold hardiness in a companion genecological experiment. In combination, the results indicate that this introgression zone is maintained by hybrid superiority limited to environments that are intermediate to the realized ecological niches of the parental species.
Habitat divergence plays a crucial role in plant speciation. It may be even more important in diploid or homoploid hybrid speciation. In contrast to polyploidy, diploid hybrid speciation is rare. Because most putative diploid hybrid species outcross, the question arises of how such species are established despite the possibility of backcrossing to the parental species. Divergence in a new ecological niche or colonization of a novel habitat might lead to reproductive isolation after initial hybridization. One example of diploid hybrid speciation supporting this hypothesis is the evolution of Pinus densata. This pine is native to southwestern China and Tibet and grows at high elevations where neither of the two parents can normally grow. At these elevations, P. densata regenerates well and forms huge areas of pure stands. Allozyme, cpDNA and mtDNA data testify the origin of P. densata through hybridization between P. tabuliformis and P. yunnanensis. Cytogenetic analysis of the hybrid genome indicates certain chromosomal rearrangements. All the data suggest a complex genetic composition and different evolutionary histories among P. densata populations. The unique territory created by the uplift of the Tibetan plateau would have facilitated the development and stabilization of the hybrid. It is difficult to distinguish among traits that facilitate habitat divergence in hybrid lineages. Recent advances in genomics and molecular cytogenetics, and combined with QTL mapping should provide more answers in revealing the adaptation mechanisms of the hybrid. In addition, gene genealogies can be used to measure the evolutionary processes of selection, migration and drift that are involved in the speciation of this hybrid pine.
Genomic Hot Spots of Genetic Differentiation Between European Sympatric Oak Species

Pedunculate oak (Q. robur L.) and Sessile oak (Q. petraea (Matt.) Liebl. are closely related species with a widely sympatric distribution in Europe. When the two species coexist in the same stands, they show clear ecological preferences and morphological differences. A whole body of literature data shows that the phenotypic differentiation is associated to an extremely low genetic differentiation. One hypothesis for the discrepancy was related to the distribution of differentiation within the genome. When numerous markers were tested, it became apparent that a rare proportion exhibited larger allele frequency variation between the two species. These results clearly suggested that genomic regions involved in species differentiation are rare and explain why earlier molecular investigations using a low number of loci proved to be inefficient. We revisited previous genetic surveys conducted in natural mixed populations with different markers systems and computed interspecific Gst (Nei’s genetic differentiation) values for all markers. Their distribution follows an L shaped curve. Moreover the Gst values were not randomly distributed along the linkage groups. In a second step we detected QTLs for leaf morphological traits exhibiting interspecific phenotypic differences. The comparison of the distribution of interspecific Gst values and QTLs revealed some interesting colocalisation. These two independent approaches confirmed the existence of hots spots of species differentiation in the oak genome.
A3-1 Müller-Starck, Gerhard*1, Fady, Bruno2, Felber, François3, Geburek, Thomas4, Klumpp, Raphael5, Konnert, Monika6, Morgante, Michele7, Ramskogler, Kurt8, Scholz, Florian9, Senn, Josef10, Vendramin, Giovanni G.11

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BIODIVERSITY IN ALPINE FOREST ECOSYSTEMS: ANALYSIS AND MANAGEMENT (BAFE, CT96-1949)

The BAFE project addresses biodiversity within and among carrier species of Alpine forest ecosystems which are increasingly threatened by environmental changes and are modified by land-use and forest management. BAFE consists of 11 partners from five countries (Austria, France, Germany, Italy, Switzerland). This project particularly focussed the study of genetic variation and its dynamics in populations of Abies alba, Larix decidua, Picea abies, Pinus cembra and Pinus mugo. Along 15 elevationally subdivided transects, 22,602 individuals were genotyped throughout the Alps. The total number of studied populations is 192 (129 adults and 63 juveniles from natural regeneration). In addition, three intensively studied plots (ISPs) have been selected at which genetic and phenotypic characters as well as the spatial arrangement of all individuals were assessed. Furthermore, five afforestation plots were established along elevational gradients by using sorted and non-sorted forest reproductive material of different geographic origin. These additional experiments will help to study long-term dynamics following reproduction and complex environmental stress. The largest intra- and inter-specific genetic inventory in high elevated forest ecosystems in Europe indicates the following: Genetic variation deviates significantly between the studied species, clearly indicating smallest values for Larix decidua and Abies alba, respectively, and maximum values for Pinus mugo. Within species, geographic patterns of genetic variation are evident. Natural regeneration tends to sufficiently represent the gene pool of the corresponding parental populations although in a couple of cases genetic erosion is evident. In contrast to the genetic differentiation among populations located at different parts of the Alps, elevational trends are the exception rather than the rule. Some examples indicate losses of genetic variation in high elevations. The study of commercial forest reproductive material reveals substantial deviations with respect to genetic diversity, to loads of inbreeding, growth parameters and survival rates. It is demonstrated that sorting of forest reproductive material affects such parameters. Generally, BAFE contributes efficiently to the assessment of biodiversity of Alpine forest ecosystems and the quantification of genetic resources. Data allow broad scale modelling with respect to reproduction, gene flow, viability selection, adaptation and survival. Results will help to better understand the role of genetic diversity for adaptation and survival, respectively, and to utilise this information in order to avoid risk situations. Important for the transfer into practice is the use of genetic criteria for recommendations concerning natural regeneration and afforestation, the declaration and management of gene resources as well as the restoration of ecosystems by means of an increases of genetic diversity. Improved reproductive material is an essential component of the genetically sustainable management of Alpine forest ecosystems.
Neutral and Adaptive Genetic Diversity of Mediterranean Conifers

Neutral and adaptive genetic diversity of Mediterranean conifers were studied in the frame of two EU contracts: MPC (FAIRCT95-0097) and FORADAPT (INCO-ERBIC18CT970 200). The general objective was to guarantee the sustainability of the Mediterranean forest ecosystem through afforestation and conservation of genetic resources. The basic scientific aims were to study the diversity of three economically important groups of conifers in the Mediterranean basin (Pinus pinaster, Pinus halepensis and Pinus brutia, Cedrus sp): Ø Characterization of the genetic variability using neutral molecular markers in a set of populations covering a wide range of ecological zones in the natural occurrence of the species. Ø Characterization of the variability in drought and frost response in the same sample of populations. Ø Approach of evolutionary processes within and among populations with emphasis on hybridisation and introgression. Ø Investigation of the ecophysiological and molecular mechanisms involved in stress response by focusing on the definition of ecophysiological early tests for drought and frost tolerance (and/or avoidance), as well as the detection of proteins and DNA probes associated with the response to drought. The applied scientific objectives were: Ø Selection and creation of tested material (stress resistance; major pests and diseases resistance) for breeding and afforestation Ø Generation of data leading to afforestation, breeding and gene conservation programmes. The projects yielded three important results. (1) Determination of Cedrus sp. and Pinus sp. seed sources adapted to specific ecological conditions after provenance and progeny tests. Special attention should be paid in the future to the risks of hybridisation between genetically close Mediterranean species, which may reduce intraspecific adaptive potential. This problem is confounded by the contrasting level of adaptive diversity between these species and the potential interaction among the level of stress and disease resistance. Improved hybrid material is interesting for wood production but the risk for native genetic resources should be considered. (2) Genetic diversity analyses using neutral genetic markers allowed describing the level and structure of genetic diversity in the whole natural range of the species. Eastern Mediterranean countries have concentrations of forest resources of considerable interest, presenting centres of high diversity. This information can be used for future conservation, management and utilisation of genetic resources. A practical application of genetic marker development and the study of diversity patterns, was the establishment of diagnostic tests for the certification of seed and plant material. (3) The underlying physiological and molecular mechanisms involved in stress response were described in growth chamber and field experiments. Variation in terms of transcript and protein accumulation as well as ecophysiological traits was observed and may provide novel early selection criteria for drought tolerant genotypes. Productive collaboration was achieved by linking 15 research laboratories and institutions in 8 countries working in the field of Mediterranean tree breeding, molecular biology, plant health and ecophysiology. This multidisciplinary approach showed its efficiency and should be enlarged and applied to other Mediterranean forest projects. In addition to the considerable amount of results and data made available for "sustainable afforestation" by MPC and FORADAPT, the need to enhance genetic resource conservation in this region emerged as the main conclusion.
GENETIC DETERMINISM OF LEAF CARBON ISOTOPE COMPOSITION FOR A PEDUNCULATE OAK FAMILY

Water use efficiency, the amount of carbon acquired by a plant per unit of water lost, can be estimated by determination of the carbon stable isotopic composition ($d_{13}C$) of plant material. $d_{13}C$ is a time integrated estimator for intrinsic water use efficiency (WUE$_i$) which can be measured on an instantaneous basis as the ratio of CO$_2$ assimilation rate over stomatal conductance for water vapour (A/g). The large variation detected for $d_{13}C$ within populations of forest tree species incited the research for a genetic basis for this variation. To this end we measured the variation of this complex quantitative trait within a full-sib pedunculate oak (Quercus robur L.) family to decompose it into mendelian-inherited genetic components (QTL analysis). We were also interested in the comparison of the within family variation of $d_{13}C$ with the variation of the ecophysiological components of water use efficiency: stomatal conductance, assimilation rate and other estimators of photosynthetic capacity. Two plantations of cuttings from the original pedunculate oak cross were available for harvesting of leaves. The first plantation, consisting of 174 siblings with on average five vegetative copies per sibling, was sampled in 2000 for $d_{13}C$ and leaf nitrogen analysis and a subsample was chosen in 2001 for gas exchange analysis. The second plantation with a larger number of siblings than the first one (207) was sampled in 2002 for $d_{13}C$ and leaf nitrogen analysis. Leaves were collected in 2002 from the first plantation and were analysed for $d_{13}C$ and leaf nitrogen. QTL analysis was performed taking account of clone and bloc effects and resulted in the detection of two highly significant QTLs for $d_{13}C$ and two highly significant QTLs for leaf nitrogen, each QTL explaining more than 15% of the detected phenotypic variance. One QTL for $d_{13}C$ and one QTL for leaf nitrogen were co-located in the same interval on the same linkage group. The gas exchange study in 2001 on 120 siblings from the same plantation was carried out over three days to investigate the dependence of leaf $d_{13}C$ on assimilation rate and stomatal conductance. Mean sibling leaf $d_{13}C$ as measured in 2000 correlated significantly with the mean sibling leaf $d_{13}C$ as measured in 2001 ($r^2 = 39\%$). A weak but significant relationship between leaf $d_{13}C$ as measured in 2001 and A/g was detected. The trend of higher A/g with less negative leaf $d_{13}C$ is in accordance with theoretical model. Stronger relationships were observed between leaf $d_{13}C$ and leaf nitrogen per leaf area or leaf chlorophyll content, two traits related to photosynthetic capacity. This is in concordance with the detected co-localisation of a QTL for $d_{13}C$ with a QTL for foliar nitrogen in 2000. In 2002, the second plantation of the same pedunculate oak cross was used for foliar $d_{13}C$ and nitrogen analysis. QTL analysis will be performed for leaf $d_{13}C$ and foliar nitrogen concentration to validate the strong QTLs found in 2000 for these traits under different environmental conditions and covering a larger number of siblings.
Existing forest stands will be subjected to climate change and will show long-term responses. The adaptability of forest tree populations is related to their potential to respond genetically or physiologically to changed environmental conditions. Additive variance in adaptive traits is the best parameter to evaluate the genetic part of adaptability. Among the various forest species of key relevance for the Mediterranean landscapes, Castanea sativa European chestnut is one of the few included in joint genetic and physiological studies aimed to assess intraspecific variability in adaptive mechanisms to water availability. The wide distribution of this species across Southern Europe and Minor Asia was associated with a large among-population genetic variability especially located in the Ponto-Caucasian region, where a climatic transition from the Mediterranean to the Euro-Siberian region is present. Physiological studies on representative populations of this region demonstrated the presence of two functionally different populations ecotypes: an eastern, wet adapted form and a Mediterranean, drought adapted one. Comparative experiments, carried out on this material, highlighted the fundamentals of adaptive mechanisms of wet and drought adapted chestnut populations. Especially the character «water-use efficiency» (WUE, the ratio of plant carbon gain to water losses) has been found to be a reliable indicator of the complex adaptive mechanisms, which differentiate the two chestnut ecotypes, the Mediterranean ecotype being characterised by lower WUE than the eastern one. Carbon isotope discrimination (D), allows estimating WUE since from the early growing stage of the plant. The present study (supported by EU project «CASCADE») deals with variability of physiological performances related to drought tolerance, among and within European populations representative of the main chestnut distribution area. Progenies from six European populations (contrasting for the mean annual temperature and precipitation of the native sites) were grown under controlled conditions, in an experiment including two watering and two temperature regimes. Significant additive variation in D and correlation between D and growth have been found. Results are discussed to highlight the potentiality of genetic-physiological approach in predicting specific adaptability to changing climate.
COMBINING QTL MAPPING WITH SELECTION STUDIES IN THE WILD: A FEASIBLE WAY TO IDENTIFY ADAPTIVE GENETIC VARIATION IN OUTBREEDING FOREST TREES?

Research into the evolution of genetic diversity in forest trees has important implications for forest management and conservation, but often new research concepts are more easily tested in annual plant species before they are applied to trees. Wild annual sunflowers (Helianthus spp.) have emerged as a powerful model system in identifying adaptive genetic variation segregating in both wild and synthetic outbred plant populations. We present results of an experiment that aimed at studying selection on adaptive phenotypic traits and the underlying quantitative trait loci (QTLs) in interspecific hybrid backcrosses between two wild annual sunflowers, H. annuus and H. petiolaris. The hybrids were transplanted into the extreme habitat of a naturally occurring hybrid species, and the increased phenotypic variance present in the hybrid lines was utilized to identify the traits involved in adaptation to the extremely saline «hybrid» habitat. A genome-wide survey of molecular markers revealed several genomic regions under selection in the wild, and several of the markers were linked to QTLs controlling uptake of mineral ions from the soil. Our results represent one step in identifying the candidate genes involved in the origin of novel adaptation in a wild annual sunflower species (salt tolerance in this example). Similar approaches are conceivable for long-lived forest trees for which provenance or field trials have been established and sufficient documentation is available. We discuss the advantages and caveats of applying this approach to trees and suggest possible alternatives, such as the utilization of adaptive genetic variation present in hybrid zones between divergent populations.
To characterize the allelic composition of a population at mutation/selection/drift equilibrium, and to investigate the role of new mutations versus the history of the population in adaptation, we simulated populations of finite size submitted to optimizing selection for a trait governed by 50-100 genes, each explaining the same part of the initial genetic variance. We measured the evolution of genetic parameters describing the populations in the course of adaptation towards a far optimum. We show that evolution occurs in three main phases: (I) Fixation or lost of the initial alleles. The genetic advance is associated with a fast decrease of the initial polymorphism and reaches an apparent plateau, (II) Linear genetic advance through fast stepwise fixations of favorable mutations. The polymorphism is minimum during this phase. (III) Mutation/selection/drift equilibrium. The different genetic parameters do not reach the equilibrium at the same time. The duration of the different phases mainly depends on the population size, mutation rate and the distribution of mutational effects. Hence, the initial genetic variation has a small impact on the long term response to selection. After about 20 generations, the initial alleles do not contribute any more to the genetic advance. The total number of genes involved in adaptation may be high, but those genes appear sequentially through time and their effect are contrasted. At a given generation there are no more than 10 polymorphic loci in a population, with a few of them explaining much of the genetic variance.
GENETIC DIVERSITY AND DIFFERENTIATION OF NEUTRAL GENES LINKED TO QTLS CONTROLLING A POLYGENIC ADAPTIVE TRAIT UNDER STABILIZING AND DIVERSIFYING SELECTION

Population genetic models predict that the levels of genetic variation at neutral loci can be influenced by natural selection at linked loci. Depending on the type of selection acting, the effects might be different. A decrease of genetic diversity and an increase of differentiation are expected around the selected locus in a subdivided population under both positive selection (genetic hitchhiking) or background selection. Diversity can also be maintained or increased at neutral sites closely linked to sites submitted to balancing selection. These questions have usually been addressed theoretically in simple models involving a trait determined by one selected locus or including very few populations. The detection of selection at the molecular level is also receiving a renewal of interest with the deluge of sequence data at candidate genes and the development of association studies. We present a simulation approach to study the genetic diversity and differentiation at neutral loci closely linked to the underlying QTLs of an adaptive trait in a metapopulation. This trait is submitted to both stabilizing selection within population and diversifying selection between populations. For this purpose, we performed simulations within the framework of the METAPOP software. The quantitative trait is determined by 10 QTLs located on different chromosomes, and each QTL is surrounded by neutral loci with increasing recombination rates. A large population is first brought to a mutation/drift/recombination equilibrium without selection and a metapopulation including 25 populations is then formed and submitted to an island migration model and different evolutionary scenarios. These scenarios consist in various intensities of stabilizing selection within population combined with different variances of optima among sets of 5 populations. The evolution of the hitchhiking effect on Gst at neutral loci is followed by testing the correlation between Gst at selected loci and neutral loci over the generations, and by estimating the linkage disequilibrium distribution along the different chromosomes. This work has important practical implications on the expected efficiency of association studies and for fine mapping strategies.
GENETIC RESOURCES IN MARITIME PINE (PINUS PINASTER AITON): ARE MOLECULAR AND QUANTITATIVE MEASURES OF GENETIC VARIATION CORRELATED?

Pinus pinaster is a conifer native to Western Europe. Breeding populations were built up in some countries of the range of the species (France, Portugal and Spain), due to going-on breeding programmes. Different provenance and progeny trials were established and quantitative traits measured: growth, stem form, survival, pest and disease resistance, amongst others. Populations from the wide-range of P. pinaster were recently screened with molecular markers in order to assess genetic diversity. Data were obtained using allozymes, mitochondrial DNA, chloroplast and nuclear microsatellites, and amplified fragment length polymorphism markers. In this study, a synthesis of the obtained results with molecular markers is provided. Moreover, the congruence between molecular and quantitative markers concerning the level of diversity and differentiation at the wide range of the species was studied. Provenance and progeny tests were used to compute quantitative measures of genetic variation and differentiation: the coefficient of variation (CV), the additive genetic variance, the narrow-sense heritability (h2) and the pairwise Mahalanobis distance among provenances. The molecular and quantitative datasets were compared by using the Kendall’s t and the Mantel’s product-moment correlations. The comparison of data from «neutral» markers (i.e. not affected by selection pressures) and from quantitative traits could be helpful to define recommendations for the conservation and management of genetic resources of P. pinaster.
ADAPTIVE VARIATION AND GENETIC DIFFERENTIATION IN MARGINAL POPULATIONS

Several broad-leaved trees that are common in central Europe have northern marginal populations in Scandinavia. This motivated us to study the amount and distribution of adaptive variation in marginal vs. central populations. In the presentation, we will ask following questions: how much there is genetic variation in marginal populations both in neutral markers and in potentially adaptive traits, how well does the amount of genetic differentiation in neutral markers describe the differentiation in adaptive traits, and do marginal populations contain genetic variation which is absent from large central populations? We try to address these questions 1) with genetic studies on the amount and distribution of adaptive variation with simple inheritance in a model species (trichome production in Arabidopsis lyrata), 2) with marker and quantitative genetic data from marginal and central populations of Acer platanoides.
PHYLOGEOGRAPHY AND POST-GLACIAL MIGRATION HISTORY IN SOME BROADLEAVED AND CONIFER FOREST SPECIES AS REVEALED BY ORGANELLE DNA MARKERS

The recent progress in molecular organelle genetics has provided insights into the phylogeography and migration history of many forest tree species. The use of different uniparentally (chloroplast and mitochondrial) inherited markers allowed investigating the levels of genetic diversity and differentiation and, in combination with pollen data, allowed refining the migration processes in the post-glacial period of various forest tree species. Based on a large set of data, relevant factors determining the actual distribution of diversity within and among species were identified: i) the location of glacial refugia in the Italian, Iberian and Balkan peninsula and the impact of the resulting migration processes and migration routes; ii) the role of life history traits, and iii) the influence of human activities on the different species. A common migration history of different forest tree species was observed and common phylogeographic patterns among species were identified. The role of life history traits (in particular seed and pollen dispersal mechanisms) and of human impact on the diversity was described and clarified. Examples of broadleaved and conifer species are provided and discussed with the aspect of different post-glacial history, life history traits and human impact. In conservation and management of genetic resources, phylogeographic studies may help to identify key regions deserving priority for conservation. They may also provide important information to develop criteria and tools for the certification of reproductive material.
PHYLOGEOGRAPHY OF NORWAY SPRUCE IN THE ALPS: COMBINED ANALYSIS OF NUCLEAR AND CHLOROPLAST MICROSATellites REVEALS GENETIC STRUCTURING AND PAST HISTORY OF POPULATIONS

The reconstruction of the past history of Alpine populations of Norway spruce (Picea abies Karst.) is addressed by means of nuclear and chloroplast microsatellite markers. Thirteen populations of 100 to 150 samples have been characterised at 8 nuclear loci and 3 chloroplast loci. The analysis of within population genetic diversity shows no relevant difference among the populations, and no traces of past genetic bottlenecks could be found in any stand. However, genetic variability is not evenly distributed over populations, as AMOVA reveals that two or three main sub-regions can be identified in the Alps. The south-western populations also carry a subset of alleles at high frequency, that are rare in the other populations, possibly indicating a different origin for these stands than for the rest of the Alpine spruce forests. Partition of genetic diversity and of populations into groups is revealed when $F_{ST}$ is used as a measure of genetic differentiation, but not when $R_{ST}$ is used, indicating that the process of population differentiation is driven by drift rather than by mutation, and constrained by gene flow. The same is suggested by the correlation of $F_{ST}$ values with geographic distances. Model-based clustering methods that use multilocus genotypes to infer population structure, and methods based on principal co-ordinate analysis, are also applied to our data set in order to reconstruct the contribution of different populations, loci and alleles to the observed pattern of diversity, and to try to reconstruct past events of population admixture and migration.
NEOTROPICAL DIFFERENTIATION OF AN ANCIENT RAINFOREST TREE, SYMPHONIA GLOBULIFERA LINNAEUS

Many Neotropical rainforest plant species are broadly distributed between Central and South America, but as yet no studies have attempted to link landscape evolution with population structure in this class of plants. To address this relationship, we examined the phylogeographic structure of the rainforest tree, Symphonia globulifera, whose appearance in the New World is documented by unmistakable fossils from the early Miocene (ca. 23 Ma, Mesoamerica) and mid Miocene (ca. 15 Ma, South America). Nucleotide variation in the nuclear ribosomal spacers (ITS) revealed three clades: (i) trans-Andes (Central America + W. Ecuador), (ii) Amazonia and (iii) the West Indies. Along with the initial colonization of the New World from Africa, our analysis shows that separate oceanic dispersals led to the colonization of Amazonia and the Pacific coast of South America. Strong phylogeographic structure within the trans-Andean clade suggests that mountain building has played a role in the regional genetic structure. Symphonia globulifera has persisted for more than four geological epochs, and its population structure reflects a landscape history that must have promoted differentiation in many other Neotropical plant taxa.
THE LOST PINES: A TEST OF THE RETREATING EDGE MODEL

Reconstruction of post-Pleistocene population dynamics is centered on colonization and retreating edge effects. DNA signatures of advancing colonization is altered by life history attributes but the retreating rear edge has been less studied. The Lost Pines, a set of scattered pine islands in central Texas, is disjunct from the larger range of Pinus taeda L.. These pine islands have a wealth of historical and prehistorical information dating back to the Pleistocene. Once a larger pine forest, the Lost Pines population appears to be a remnant contracting to its present-day distribution. Contraction may have occurred as a consequence of harsh subtropical climate changes during the Holocene. The Lost Pines population is thus a model for testing the genetic consequences of the retreating-edge effect in a woody perennial species. Our objectives were 1) to test for one versus two Pleistocene refugial populations of P. taeda using nuclear microsatellites, 2) to test for bottleneck events in the western retreating edge, 3) to use simulated data to pinpoint prehistoric events which paralleled the bottleneck(s) events. Using nuclear microsatellites and a haplotypic analysis of the species’ range, we have detected one or more bottleneck events in the Lost Pines. The genetic implications of the bottleneck and genetic diversity patterns are discussed with respect to several genetic models, metapopulation dynamics and the life history of the species. Constructing hypotheses for pinpointing the timeframe of the historic events causing the bottleneck has been an ongoing collaborative effort with archaeologists, ecologists and geologists.
MAY INCLUSION OF TRAIT DIFFERENCES IN PHYLOGEOGRAPHIC ANALYSIS ALTER OUR VIEWS?

What happens to phylogeographic findings when new lab techniques discern more allelic variants than the old methods? Can this feign phylogeographic differences that are merely due to overrating of the differences between the new variants? How may this affect conclusions on adaptive differentiation? Does comparison of phylogenies based on microsatellites with and without consideration of differences in tandem repeat number give clues about how microsatellite alleles mutate? To answer such questions, phylogenetic analysis must reflect not only differences in the frequencies of the genetic types but also differences between the types themselves. Real data will be reexamined in the light of these questions.
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THE FOSSILVA PROJECT (DYNAMIC OF FOREST TREE BIODIVERSITY: LINKING GENETIC, PALAEOGENETIC AND PLANT HISTORICAL APPROACHES)

The aim of this project is the study of past biodiversity in fossil tree material across a network covering western and central Europe, involving an association of palaeobotanists working on natural archives (lake sediments, peat bogs, buried trunks) and plant population geneticists. The potential for the recovery and study of fossil DNA in tree remains is explored. In one task, a molecular atlas based on cpDNA sequences of woody taxa is developed to assist in the taxonomic identification of any woody fossil remain. Some species are then the focus of more work in another task: The targeted forest trees are 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), 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 need improvement and validation. Our 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. This strategy is developed in order to help identify the status (natural, artificial or mixed) of present day fragmented forests and thus will contribute to improve their management. The project has contributed in the launching of a macrofossil database and in an improvement of the European pollen Database, leading to a better understanding of the postglacial migrations of the selected trees from their glacial refuges; the comparison with the phylogeographical approach leads to consistent progress on the understanding of their present day setting. The extraction of fossil DNA from plant material is a difficult task and a long period of calibration suggests that conservation of DNA is very erratic. The first positive results illustrate the potential and the limits of this approach.
This contribution presents the results that were obtained within a EU supported research project gathering efforts of 13 research stations from 10 countries (FAIROAK project). Synthetic maps of chloroplast DNA haplotypes were constructed in white oaks at a European scale. The maps are based on a total number 2673 populations (12714 trees) that were collected in eight different species (Q. canariensis, Q. faginea, Q. frainetto, Q. macranthera, Q. petraea, Q. pubescens, Q. pyrenaica, Q. robur) distributed in 22 different countries. A common strategy for molecular screening, based on restriction analysis of four PCR-amplified cpDNA fragments, was used to allow comparison among the different studies. 42 chloroplast variants were detected during this survey and their phylogenetic relationships were described. These variants clustered in six cpDNA lineages, which have quite separate geographic distributions along a longitudinal gradient. A low level of diversity within population but strong differentiation among populations and clear-cut geographic patterns have been revealed during this survey. A phylogeographic pattern was apparent, with related haplotypes from the same lineage having broadly similar geographic distributions. Simultaneously, geographic maps of fossil pollen deposits were constructed at 500 years intervals between 15000BP and 6000BP. These maps are based on 600 pollen sequences available from the European Pollen Database (EPD, 483 sequences), the Alpine Palynological Database (ALPADABA 50 sequences) and about 70 sequences supplied for use in the project or digitised from published pollen diagrams. Three areas of southern Europe have been identified as refugia: southern Iberian peninsula, southern Italian peninsula and the southern Balkan peninsula. The spread of Quercus took place in two steps. First, in the late glacial interstadial (13 to 11 ka BP) Quercus spread to the central European mountains from these refugia. Second, with the stabilisation of a climate favourable to deciduous trees species in the
Holocene, the oak spread into northern Europe, rapidly into the north-west, and more slowly into the centre and east, due to physical barriers. By approximately 6 ka BP, the deciduous oak had reached its maximum extension in Europe.

The distribution of haplotypes and the palynological information available were used to infer colonisation routes out of the ice-age refugia. In western Europe, in particular, movements out of the Iberian and the Italian peninsula are clearly apparent. Some movements resulted in the exchange of haplotypes among refugia, during the present interglacial and probably also during earlier cycles. This has lead to parallel colonisation routes for phylogenetically divergent haplotypes, limiting somewhat the phylogeographic structure. Colonisation routes may have been initially constrained by the ecological characteristics of the species hosting each chloroplast variant. We suggest for instance that two oak species distributed in the north of the Iberian peninsula (Quercus petraea and Q. robur) are recent postglacial immigrants. Altogether, the conclusions in terms of the location of ice-age refugia and of the colonisation routes derived at with the molecular information and with the fossil pollen data appear to be largely compatible.

The association between chloroplast DNA variation and nuclear controlled traits (phenotypic traits and gene markers) was tested in 16 provenance tests and two nuclear diversity surveys in deciduous oak species (mostly Q. petraea). All over assessments of 62 different phenotypic traits (traits*tests), 8 isozyme loci and 31 RAPDs loci were used for the analysis. Among the 62 phenotypic traits, only 7 exhibited significant associations with maternal lineages using ANOVA (mostly growth traits), and 6 using Mantel's test. There were stronger associations with gene markers. The existence of cytonuclear desequilibrium was shown by the significant differences of allozyme frequencies among the four maternal lineages (at least one allele for each locus). Finally associations were also found between levels of nuclear diversity and maternal lineages. These results suggested that local selection pressures acting on the installed populations and pollen flow erased progressively the initial differentiation that existed among the three refugial zones after the last glaciation. At the same time, chloroplastic divergence remained still untouched. As a result, there is no association any more between chloroplastic divergence and phenotypic traits, but there is still some association with gene markers that are less affected by selection.
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TREE MIGRATION CAPACITY UNDER RAPID CLIMATE CHANGE: EVIDENCE FROM THE EARLY HOLOCENE

Fossil pollen data are believed to demonstrate that tree populations can migrate rapidly in the face of climate change, at rates of 100 to 1000 m yr⁻¹. We used chloroplast DNA surveys to show that the geography of post-glacial range expansion in two eastern North American tree species differed substantially from pollen based reconstructions and from patterns emerging from European molecular studies. Our molecular evidence suggests that American beech (Fagus grandifolia) and red maple (Acer rubrum) persisted as low-density populations close to the Laurentide Ice Sheet, at higher latitudes than interpreted from pollen. Because populations were much further north than previously thought, postglacial migration rates were slower than those estimated from fossil pollen. Our estimated rates of <100 m yr⁻¹ are consistent with model predictions based on life history and dispersal data. This new evidence suggests that past migration rates were substantially slower than the rates that will be needed to track 21st Century warming.
GENETIC CONSEQUENCES OF FOREST POPULATION DYNAMICS INFLUENCED BY CLIMATIC VARIABILITY IN THE WESTERN U.S.

Most analyses of genetic data assume relatively stable environments, at least over the past 10,000 years. However, recent advances in paleoclimatology have indicated substantial climatic variability over this period. We first review what is known to date about climatic variability over interannual to millennial scales, giving an example of the influence of these cycles on vegetation dynamics in the Great Basin. We next discuss the impact of these cycles on tree population dynamics: 1) Effects of interannual and decadal variation on Pinus albicaulis population dynamics over the past 600 years. Since the end of the Little Ice Age, 150 years ago, tree line for upright forms of this species has been increasing in pulses driven by multidecadal phase shifts. 2) Millennial effects on demographic structures and histories in P. flexilis over the past 3000 years. Northern Sierra Nevadan and cis-Great Basin populations have been extirpated from southern aspects and expanding onto northern ones. 3) Multimillennial influences on the population histories of Californian Quercus (oak) species over the past 50,000 years. The palynological record indicates that oaks in California were rare during glacial maxima and common during the interglacials. Thus current population sizes are currently anomalous. We then consider the effect of these histories on known genetic structures. Our data suggest that migration by seed has greater influence on observed genetic structures than migration by pollen. This observation is consistent with recent theoretic models on the response of populations to environmental and climatic gradients (Pease, et al. 1989, Garcia-Ramos and Kirkpatrick 1997, Kirkpatrick and Barton 1997), which also indicate that migration is more important than genetic factors in population responses to gradients.
YEAR AND TREE EFFECT ON REPRODUCTIVE ORGANISATION OF CEDRUS ATLANTICA IN A NATURAL STAND.

Reproductive parameters (male and female flowering intensity, pollen germination, seed yield and seed quality) have been analysed on Cedrus atlantica individuals for several years in two adjacent stands in Algeria. The results indicated an important variation for each parameter with a strong year effect on male and female contribution in addition to a tree effect and a year x tree interaction. The hypothesis formulated is that fertility differential among trees should lead to a particular spatial structure where contribution to stand recruitment should be ensured by the most fertile trees. We expect an impact on the genetic diversity resulting from two processes: the variation of individual fertility parameters decreases the effective population size, whereas the year x tree interaction limits the impact of environmental (year) stochasticity when recruitment cumulates over several years.
Small scale spatial patterns of tree species distribution is expected to depend mostly on 1. the spatial heterogeneity of environmental conditions, 2. the modes and patterns of seed dispersal. When the effect of environmental heterogeneity can be neglected, species with limited seed dispersal are expected to aggregate more than species with extensive dispersal abilities, so that dispersal distances should be inferable from the spatial distribution of the individuals of each species. Adapting theoretical results of isolation by distance models from population genetics, we can describe what is the expected pattern of species distribution according to seed dispersal distances, assuming a neutral hypothesis (all individuals are competitively equivalent, regardless of the species) and independency of dispersal events. Simulations show that estimates of seed dispersal distances can then be obtained with fairly good accuracy. The spatial patterns of species distribution was investigated in a tropical forest of Cameroon and seem to conform to the predicted patterns for most species. For some species, a significant impact of environmental heterogeneity could also be detected.
LOCAL PATTERNS OF RELATEDNESS AND BI-PARENTAL INBREEDING IN EUCALYPTUS GLOBULUS

Seed dispersal is limited in Eucalyptus globulus and consequently, there is potential and some evidence for family structuring to occur in native populations. However, selection against biparentally inbred individuals could cause the degradation of family structure over time. Knowledge of paternally mediated gene flow (pollen movement) in native populations is critical in unraveling the dynamics of this system. Genetic relatedness and pollen movement between individuals within a native E. globulus population were investigated. In a natural population, 14 microsatellite loci were used to genotype 36 mature E. globulus trees within a 40m x 40m quadrat and an additional 50 trees in groups of 10 separated from the quadrat by 25, 50, 100, 200 and 400 metres. Pairwise relatedness values were obtained for all 86 trees. An average of 30 seed was collected and germinated from 5 trees within the original quadrat. These progeny were subsequently genotyped, using the six most informative microsatellite loci, and paternity analysis carried out. Where pollen parents were identified with sufficient confidence, the relatedness values between mother and father were used to estimate the occurrence of bi-parental inbreeding within this population. Aside from answering important ecological questions, the findings of this study will have important implications for commercial breeding programs of this species that are based on open-pollinated seed.
Macadamia integrifolia is a vulnerable, sparsely distributed, sub-tropical rainforest tree of the eastern coast of Australia. Today, the species is found mostly in small patches within cleared or disturbed landscapes. In this study we examine the structure and dynamics of three isolated populations to investigate their long-term viability. Dominant and co-dominant RAF (randomly amplified DNA fingerprinting) markers were used to genotype all individuals from three populations in the Brisbane region. Population sizes were 211 (64 adults), 142 (5 adults), and 113 (51 adults). Spatial genetic structuring was observed to 75 and 100 m, suggestive of limited gene flow. There is also evidence that landscape was an important determinant of within population genetic structure. Significant differences between the spatial structure of adult and progeny cohorts were also observed. While the adults exhibited strong positive structure, no evidence was found for spatial structuring in the progeny cohort. These results suggest that patterns of gene flow in M. integrifolia populations have been altered by habitat fragmentation. Data will also be presented for patterns of gene flow assessed via paternity analyses of fruit collected from these populations. The implications of these results for the viability of these isolated populations are discussed.
Sufficient genetic diversity as well as the presence of special adaptive genetic variants found among seeds are generally considered as basic prerequisites for the genetic adaptability of the next generation. Both, however, may spatially vary and control a spatially differential success of natural regeneration. In a small isolated stand of beech (Fagus sylvatica L.), beech nuts were collected in (more than 250) circular sample plots with diameters of 40 cm placed under seed trees at a distance of 3 m from the stem. These beech nuts were investigated at enzyme gene loci. For each plot the gene pool diversity, the mean heterozygosity at the enzyme gene loci, as well as relative frequency of particular enzyme variants are determined. Gene pool diversity and mean heterozygosity substantially vary on a small scale. The genetic equipment of the adult beech trees plays a particular role which is eventually covered by stand and environmental characteristics affecting gene flow via pollen and seeds. Shelter (lee) effects by neighbour trees are expected to reduce genetic variation. Spatial patterns are also observed for some special enzyme variants which proved adaptive relevance in former experiments. Maps illustrating the spatial variation of genetic parameters are presented and compared with the density and genetic structures of natural regeneration.
ADAPTATIONAL CHARACTERISTICS OF AUTOCHTHONOUS POPULATIONS - CONSEQUENCES FOR PROVENANCE DELINEATION

The autochthony of source populations is a major concern in provenance research and regulations. Autochthony is closely associated with the notion of adaptedness, which entails the special features of the environment to which a population is adapted. These environmental features in turn determine the delineation of provenance regions that encompass autochthonous populations. Degree and scale of spatial and temporal environmental heterogeneity or variability define categories of adaptational requirements, which must be matched by the mechanisms of the genetic system on the species and population level. Consideration of these categories may affect the evaluation of autochthony in provenance tests and regulations. Among others, this evaluation will refer to problems of detecting adaptational processes in provenance trials through genetic analyses of diebacks, to information obtainable from provenance tests on the category of environmental heterogeneity to which the source population is adapted, and to opportunities of including categories of enviromental heterogeneity into the delineation of provenances. On which scale this approach can be applied to existing field trials remains to be answered.
GENETIC VARIATION AMONG AUTOCHTHONOUS AND PLANTED POPULATIONS OF PICEA ABIES FROM THE VOSGES MOUNTAINS IN REFERENCE TO OTHER FRENCH POPULATIONS ASSESSED BY RAPD AND MTDNA MARKERS.

In the Vosges mountains, small autochthonous stands of Picea abies are disseminated among populations massively planted at the end of the XIXe century or after the Second World War from allochthonous or unknown origin. This situation is quite different from the other French genetic resources of Norway spruce. In this study, we report a first description of the genetic variation among four autochthonous Picea abies populations and one planted population of the Vosges mountains in comparison with three other French autochthonous populations from the Jura and Alps mountains and one seed orchard using RAPD and mtDNA markers. The RAPD study was based on a genotypic analysis by assessing alleles frequencies from dominant markers data and on phenotypic analysis. 109 RAPD markers generated by 30 primers were scored. Levels of within population genetic diversity estimated RAPD did not varied substantially among the French populations of Norway spruce. The two level AMOVA, as well as the FCA, revealed that the greater differentiation was observed between three groups: Vosges, Alps/Jura and Southern Alps. UPGMA clustering of populations based on Nei's genetic distance revealed many similarities with FCA. In the Vosges, autochthonous and planted populations are quite similar. The analysis based on mtDNA led to further conclusions. One major mtDNA haplotype was found in the autochthonous populations investigated. This major haplotype was also mainly found in population from the Alpine domain in Europe. These results are concordant with the postglacial history of Picea abies at the western margin of this natural area. In contrast, the planted population showed a high degree of polymorphism (3 haplotypes) leading to support an transfer of material from east of Europe in the late XIXe century. The potential use of these markers in terms of delimitation of Picea abies genetic resources in France and the following of the genetic material is discussed.
VERIFICATION OF THE MODEL OF GENETIC IMPROVEMENT OF SCOTS PINE IN POLAND

Paper presents a concept of studies taken up to verify the way of genetic improvement of Scots pine in Polish State Forests through the selection of populations described phenotypically. The aim of these studies is to answer the question whether the genetic variability of Scots pine selected seed stands is transferred to their progenies regenerated artificially. Very early results based on isoenzyme studies indicate a genetic variation level in such progenies similar to that observed in the maternal population (i.e. seed stand). However, some rare alleles probably are not transferred from the maternal population to the progeny. On the other hand, new alleles, which appear in the progeny populations seem to be a result of outside pollen flow to the maternal population. Progenies of the same maternal population originating from different seed years significantly differed in the set of rare alleles. Other currently going on project is concerned with the testing of genetic variation level in progenies of both clonal and seedling seed orchards compared with the progenies of economic stands of Scots pine in Poland.
LOSS OF ALLELES DUE TO RESISTANCE BREEDING: THE CASE OF CYPRESS

Various individuals in six populations of Mediterranean cypress (Cupressus sempervirens) were selected for resistance against the imperfect fungus Seiridium cardinale. The six collection of resistant clones and their base populations were surveyed at several isoenzyme gene loci. A total of 140 adult trees and of 109 clones were genotyped at six gene loci. The comparison yielded information on changes in genetic variation due to artificial selection. Genetic structures of most clone collections were similar to their base populations. Nevertheless, the number of rare alleles among the resistant clones had consistently decreased, although the numbers of sampled trees were similar to those of the clones. Possible implications for breeding strategy are discussed.
EFFECTS OF SELECTIVE LOGGING ON FOREST STRUCTURE AND GENETIC DIVERSITY OF SHOREA CURTISII DYER EX KING (DIPTEROCARPACEAE)

Effects of logging under the Malayan Uniform System (MUS) and Selective Management System (SMS) on forest structure and genetic diversity of Shorea curtisii Dyer ex King were determined for different age cohorts after different time periods of logging, viz. immediately after logging, after 2 ½ years of logging and after about 50 years of logging. Three forest reserves in Peninsular Malaysia, i.e. Ulu Sedili Forest Reserve in Johor (Compartment 118), Serting Tambahan Forest Reserve in Negeri Sembilan (Compartment 40 and 48) and Panti Forest Reserve in Johor (Compartment 69) were selected for the study. The effects of logging on forest structure was carried out based on enumeration of all trees ≥ 1 cm diameter at breast height (dbh). The total reduction in mean basal area for trees ≥ 1 cm dbh for both logged stands was significantly different (p < 0.05), i.e. 50.9% in Compartment 118 and 51.8% in Compartment 48 under SMS. In Compartment 118 and 48, all trees more than 60 cm and 75 cm dbh, respectively, were felled in a single selective cutting under SMS. The mean basal area and tree densities for seedling and sapling classes (< 5 cm dbh) were reduced to one-half of the original stand in Compartment 118 after logging, meanwhile an increment of 24.3% was observed in Compartment 48 after 2 ½ years of logging. This indicates that the logging operation favoured the growth of seedlings and saplings. The net loss in trees was offset by incremental growth in surviving trees. In addition, good regeneration was observed in the regenerated stand of Compartment 69 (logged under MUS) with 28.5% and 20.5% relatively more, respectively in basal area and tree density for seedling and sapling class (< 5 cm dbh) compared to Compartment 118 before logging. The high negative correlation of basal area with relative disturbance index (RDI) was observed in Compartment 118 and 48 based on botanical name or native name, indicating that the degree of disturbance was affected by the type and magnitude of disturbances in each of the localities in the compartment. This further implies that a logging operation in the forest is not a homogeneous activity.

The initial step in genetic study involved optimization of the PCR protocols used in simple sequence repeats (SSRs) and direct amplification of minisatellite-region DNA – PCR (DAMD-PCR) analysis for S. curtisii. Samples of S. curtisii collected from Compartment 118 before logging were used to evaluate the correlation coefficient of SSR and DAMD-PCR markers. The genetic distance matrix generated from SSRs data was significantly correlated with DAMD-PCR genotype data (r = 0.990, p < 0.05), indicating a similar genetic structure of S. curtisii being depicted by both markers types among the age cohorts tested.

Genetic diversity of S. curtisii from different age cohorts, i.e. seedlings, saplings and adult trees were determined using six SSR loci. Standard and non-traditional genetic diversity measures were used to define genetic diversity changes caused by logging. In general, the reduction of genetic diversity measures were in the following sequence: saplings < seedlings < adults. The genetic diversity levels of seedlings and saplings after a single logging event under SMS and the MUS are still considerably high or equivalent with the genetic diversity levels detected in the unlogged stand. The hypothetical gametic diversity (\(V_{gam}\)) and latent genetic adaptive potential (LP) increased after logging in seedlings and saplings, suggesting that seedlings and saplings have the potential to produce genetically diverse gametes when mature with the capability for colonisation or adaptation to environmental changes in the
long-term. However, logging caused loss in genetic diversity and long-term evolutionary potential of adult trees in the logged stand. There was a significant (p < 0.05) loss of alleles (50.0%) detected in adult trees immediately after logging in Compartment 118. Low levels of $V_{gam}$ and LP in adult trees suggest that the ability of this gene pool to adapt to changing environmental conditions may have been compromised. Nevertheless, the losses in genetic diversity of adult trees may be compensated for (as shown in this study) by an existing good seed or seedling bank in the forest management unit (FMU) or by migration from nearby undisturbed forest areas. Hence, it is crucial to have adequate buffer zones whilst at the same time leaving behind sufficient undamaged good quality adolescent or bigger trees to ensure good regeneration in the residual stands. All the age cohorts examined in this study showed positive fixation indices ($F_{is}$) except seedlings from unlogged stand (UL-C40), suggesting a general excess of homozygotes. Deficiency in heterozygote was also contributed by the presence of null alleles at SSR loci where an average of 15.8% was detected in S. curtisii.

DAMD-PCR analysis was carried out for the same set of samples with SSRs analysis from Compartment 118. Genetic diversity parameters were estimated based on 33 reproducible fragments/DAMD-PCR loci. Two approaches were used to estimate the genetic diversity parameters, i.e. phenotypic method using Shannon's diversity index (S) and genotypic method with null-allele frequency corrected for deviation from Hardy-Weinberg equilibrium with SSR markers. In general, the reduction of genetic diversity parameters were in the following sequence: saplings < seedlings < adults. There was a significant loss (p < 0.05) of S, total number of alleles detected and mean expected heterozygosity ($H_e$) in adult trees after a single selective logging event under SMS. This result was concordant with the results revealed by SSR markers in terms of allelic richness indicating most probably good genome coverage of both markers. Therefore, in general, both DAMD-PCR and SSR markers are sensitive in monitoring genetic diversity changes or genetic erosion.
POLLEN MOVEMENT UNDER ALTERNATIVE SILVICULTURAL PRACTICES IN NATIVE POPULATIONS OF SCOTS PINE (PINUS SYLVESTRIS L.) IN CENTRAL SPAIN

As conservation genetics is integrated into multipurpose forest management, questions regarding the genetic effects of silviculture arise. Careful exploitation regimes, using natural regeneration, could preserve genetic resources within commercially important species, both in reserves and in exploited areas. We investigated the effects of two natural regeneration methods, shelterwood and group selection cutting, on subsequent pollen movement and mating system in four native stands of monospecific Scots pine (Pinus sylvestris L.) in the Guadarrama Chain of central Spain. Using the newly developed TwoGener analysis, we estimated an average pollination distance (d) ranging 17-22 m, and a high effective number of pollen donors (Nep 70). We found no significant silvicultural effect on pollen dispersal, although a trend was observed toward increasing pollination distance and larger effective number of fathers after cutting. Considering the high conspecific density of the studied stands (80 to 315 trees per ha), pollen dispersal estimates seem consistent with values from other studies, using other methods. Mating system analysis, using the MLTR mixed-mating model, showed high outcrossing rates for all four stands (tm = 0.93 - 0.99), but failed to show significant effects of stand thinning, although slight increases of the outcrossing rate and the apparent rate of consanguineous mating (tm - ts) occurred after cutting. Results suggest that the pollination system of Scots pine is resilient enough to preclude a negative impact of natural regeneration cutting. Indeed, a reduction in stand density can lead to an increase of genetic diversity within the offspring of single mothers. From the pollination point of view, normal Scots pine silvicultural systems seem compatible with genetic conservation purposes in central Spain.
Little is known about the impact of logging on the spatial genetic structure of trees. In tropical forests logging results in the removal of individuals of large diameter which are in general reproductive trees. Both the density and the spatial distribution of the reproductive individuals are modified, especially for species which are naturally at a low density. Microsatellite nuclear markers were used to assess genetic diversity in Sapelli, a tropical tree from the Congo Basin. Two stands were studied in the Cameroon rain forest: one still exempt from logging activity until this year to investigate immediate effect of logging, and one logged between 1958 and 1974 to assess the long term effects of logging. We compared the spatial genetic structure between adult trees (diameter greater than 50 centimetres) and young adult trees (diameter less than 50 centimetres) at each site, and the genetic diversity at both sites. Both sites showed high levels of genetic diversity (values of the Nei diversity index 0.33) and the same levels of deficiency in heterozygosity ($F = 0.09$). Equivalent allelic frequencies were observed. Adult trees presented a weak but significant spatial structure of genetic diversity, assumed to be due to limited seed dispersion and extensive pollen-mediated gene flow: individuals distant of less than 200 meters from each other were genetically related in both stands. It seems that no spatial structure was observed for young adult trees in the site logged 30 years ago.
DEMO-GENETIC PROCESSES AFFECTING INTRA-SPECIFIC DIVERSITY IN A SCATTERED FOREST TREE

Though dominated by the so-called social tree species, most temperate forest stands are mixed: thus, the genetic diversity and structure of a given species will also depend on interspecific processes at the community level, and on anthropogenic processes affecting other species. We considered here the case of a low-density tree species (the wild service tree, Sorbus torminalis L. Crantz) scattered within oak stands of Rambouillet forest. First, we showed that the spatial organization of the wildservice tree genetic diversity reflects past logging events. Then, using an individual-based simulation model, we studied how different demo-genetic processes (seed dispersal, inter-specific competition, forest logging) can shape spatial genetic structures. Finally, we discuss the importance and the consequences of the anthropogenic component of spatial genetic structure.
CO-ORDINATION FOR THE CONSERVATION OF ELM GENETIC RESOURCES IN EUROPE

Throughout Europe, the three indigenous elm species are submitted to the strong pathologic pressure exerted by Dutch elm disease. In the case of the White elm (Ulmus laevis Pall.), the destruction of riparian forests has caused substantial habitat and population fragmentation, leading to risks of genetic drift. Consequently, the ‘Noble Hardwoods’ network of the pan-European EUFORGEN programme has promoted strategies for the longterm conservation of elm genetic resources in Europe. These strategies associate in situ dynamic conservation measures, in order to maximize the genetic diversity among conservation populations, and ex situ methods, especially when populations are endangered in situ. The financial support of the European Union to project RESGEN CT96-78 has permitted to co-ordinated and rationalize the ex situ conservation of elms. The project, which involved partner institutes in 9 countries, aimed at a better evaluation, conservation and utilization of the existing collections of native elm clones. Main achievements are the establishment of a database, characterization of genetic diversity using molecular markers, complementation and rationalization of the existing collections, development of cryo-preservation techniques, establishment of a longterm core collection, and identification of clones of interest for breeding and prudent use in the reconstruction of countryside hedges. The protocols and methods developed in the project will be disseminated beyond the boundaries of the EU through the ‘Noble Hardwoods’ network. Co-ordinated initiatives are now needed to define and rationalize in situ dynamic conservation measures in a European perspective. In particular, research work focused on intensively studied conservation populations would permit to assess the amount and effect of gene flow and provide sound bases for the definition of practical guidelines for the management of conservation stands.
GENETICS AND CONSERVATION OF TWO RELIC TREE SPECIES FROM THE MEDITERRANEAN REGION: ZELKOVA ABELICEA AND Z. SICULA

The conservation of biodiversity is among the most important responsibilities of the international scientific community in the frame of the environmental protection. Under this respect, rare and scattered species, and species whose distribution area was severely fragmented and reduced, are particularly threatened. Human impact on the natural environment intensively characterised the last thousands of years, particularly in the Mediterranean area. The development of agriculture progressively caused the modification of land use, changing forest sites in agricultural territories. Modifications of the Mediterranean environment are the result of persistent human exploitation during different periods of time. The destruction of the deciduous forests and their replacement by evergreen vegetation (macchia) occurred mainly during the younger Bronze Age, the Hellenistic period and the Greek-Roman period; a further increase in forest destruction took place during the Middle Age. However, some relics of the previous vegetation survived in ecological niches because of particular micro-environmental conditions. In the Mediterranean regions, several environmental niches preserved part of an almost extinct tree flora and have therefore significant biological value. The conservation of biodiversity implies in this case both the conservation of genetic resources of rare and relic tree species, and the conservation of threatened habitats. Two European species belong to the genus Zelkova: Z. abelicea Boisser in the island of Crete and Z. sicula Di Pasquale, Garfì and Quézel in Sicily. Together with Z. carpinifolia from Caucasus, the two Mediterranean Zelkova form the south European and Near East complex of the genus, whereas the collective species Z. acuminata Planchet represent the genus in southeastern Asia. Zelkova is believed to have disappeared from continental Europe during last glaciation: fossil pollen data registered the last expansion of the genus about 75000 years BP. After that period, the genus progressively decreased and finally became almost extinct; it remains now as endemic only in the two Mediterranean islands that acted as glacial refugia. Chloroplast (trnL) and ribosomal (ITS2) sequences, chloroplast DNA markers (PCR-RFLP and SSR), and nuclear DNA markers (ISSR and AFLP) were analysed in the two species. The analysis of the plastidial trnL intron and of ITS2 ribosomal sequences revealed their divergence from the related species Z. carpinifolia, widespread in the Caucasian region; one base substitution in the trnL intron was detected between the two Mediterranean species, thus suggesting their recent separation. Chloroplast markers showed: i) an evident genetic differentiation between Z. sicula and Z. abelicea, the two species being characterised by different haplotypes, ii) no variation within the populations. The analysis of nuclear markers (ISSR and AFLP) showed genetic variation within the Z. abelicea population but not within the Z. sicula population. This species, existing as a unique population, revealed to be represented by a unique genotype, which is not present in the Z. abelicea sampling, thus indicating that an extreme reduction of genetic variability characterises Z. sicula. Based on this result, we can assume that agamic propagation is the reproductive strategy of this species in its present environment, unfavourable to the development of regular and functional flowering structures. Molecular analyses indicate that the segregation of the two Mediterranean relic species might have occurred as a consequence of the strong reduction of their distribution and the following geographic isolation. The priorities for conservation programs are discussed in the light of the different genetic resources represented by the two taxa.
IMPORTANCE OF CONSERVATION OF THE HAIRY TYPE OF POPULUS NIGRA IN THE CHANGING CLIMATIC AND ENVIRONMENTAL CONDITIONS

The hairy type of black poplar (P. nigra) grows along the Neretva river (Bosnia and Herzegovina) considerably differently from the black poplar in the riparian populations along the rivers in Croatia (Danube, Drava and Sava river). This rare hairy type of P. nigra is considered to be a tertiary relict and as a xeromorphic form in the submediterranean region was compared with typical European black poplar and with P. deltoides using discriminant analysis. The analyzed species differ significantly in all five morphological traits. The role of the hairy black poplar in the conservation of the forest genetic resources and in the effects of climatic changes were studied.
CONSERVATION AND REINTRODUCTION OF RARE FOREST SPECIES: AN EXAMPLE FROM WILD GRAPEVINE (VITIS VINIFERA SUBSP. SYLVESTRIS (GMELIN) HEGI) IN ALSACE.

Wild grapevines (Vitis vinifera subsp. sylvestris) are autochthonous liana which used to be frequent in the European forests. Nowadays they have become rare and are only found in few alluvial and colluvial forests. Direct and indirect human impact on its habitat lead to its rarefaction and since 1980, the IUCN has added it to the list of endangered species. In some regions, cultivated and wild subspecies have coexisted for more than 10000 years. However it seems that there are few if any crossings between them and molecular techniques enable their identification. At the present, in Europe the majority of sites are located in floodplain forests (73 %). At the adult stage, the wild grapevine is heliophilous and it is mainly found in edges (69% of the studied cases) or gaps. Along the Rhine valley, there are presently seven localities containing one to six individuals. The biggest population is on Ketsch Island (Germany), where about 20 individuals remain. In the nineties, several experimental reintroductions were performed independently in France and Germany. Unfortunately, at that time no preliminary study on the ecology and growth strategy of the wild grapevine was available. Therefore most of the individuals were planted in unsuitable places and died rapidly (70% to 90% of death after 10 years). Most of the individuals planted in good conditions of light and humidity are still alive and healthy. This study shows that the reintroduction of wild grapevine at a bigger scale is possible. It also highlights the fact that ecological and genetic studies should be a preliminary step in program of conservation and reintroduction of rare plant species.
CAPTURING ALLELIC DIVERSITY IN EX SITU COLLECTIONS OF WIDESPREAD SPECIES: DOES SAMPLING STRATEGY MATTER?

With the aim of understanding the importance of sampling strategies for capturing allelic diversity in widespread species, we have studied the allele frequency distribution in natural populations of Sitka spruce (Picea sitchensis (BONG.) CARR.), a conifer that occupies a narrow belt stretching 3000 km along the Pacific coast of North America. A two-way classification was devised in which populations were classified as either core or peripheral based on ecological conditions, and continuous or disjunct based on distribution. Needle tissue was collected from a total of 1600 trees in eight distinct populations covering the entire natural range of Sitka spruce. We then used eight cDNA-based Sequence-Tagged-Site(STS) primers to detect the patterns of genetic diversity and distributions of rare and common alleles in the populations sampled. Observed heterozygosity (Hobs) was highest (0.57 ± 0.08) in a core and continuous population and lowest (0.45 ± 0.09) in peripheral and disjunct population. This suggests reduced genetic variation in peripheral and disjunct populations. Similar trends were observed for expected heterozygosities (Hexp) (0.60 ± 0.09) in core and continuous population and 0.53 ± 0.07 in a peripheral and disjunct population. Ninety-seven percent of the total observed genetic variation was within populations. The same alleles, whether locally common or rare, tended to be distributed throughout the range of the species. As might be expected, the observed allele frequency distribution was U-shaped in all the eight populations examined and generally agreed with the mutation-drift theory. Common, widespread alleles are easily captured irrespective of sampling strategy. Capture of rare, widespread alleles is accomplished by more intensive sampling within populations, while rare, localized alleles require intensified sampling within and among populations. Implications for the ex situ gene conservation of widespread species including Sitka spruce are discussed. Key words: Picea sitchensis, Sitka spruce, allelic diversity, rare alleles, sampling strategy, ex situ gene conservation
In situ reserves can be a very efficient means of managing and protecting genetic diversity in tree species if the reserves have the appropriate size and spatial distribution. The idea behind this «coarse filter» approach to gene conservation is that by providing spatial coverage for landscape level units we will automatically cover genetic variation as well. We use census information on the population size and distribution of tree species to assess the level of protection needed. Based on 30,000 botanical inventory plots from the Provincial Ecology Program in British Columbia we modeled the abundance and distribution of 48 tree species in British Columbia. Then, we used geographic information systems (GIS) for analysis of spatial threats (such as forest harvesting or land conversion), and gap analysis (such as lack of protection in a particular region). We find that species fall along a broad risk continuum. Many tree species in British Columbia are very common and virtually ineradicable, while others have been reduced to a few populations in some regions. This analysis helps to allocate resources for subsequent conservation efforts efficiently, and to assess the need for genetic data on different species.
Maintaining the biological diversity of an ecosystem depends upon sustaining the genetic diversity of the species comprising that ecosystem. Dominant trees are major determinants of the characteristics of other flora and fauna of forest ecosystems and, if their genetic diversity is compromised or lost, those effects are felt throughout the entire system. This relationship is especially critical for sustaining biological diversity in tropical rainforests of Southeast Asia, where many commercial hardwood species are threatened or endangered because of exploitative logging, land conversion, fires, and other factors. Governments of several ASEAN nations have recognized the importance of conserving the genetic diversity of forest trees when developing their national plans to conserve biological diversity. These governments have supported research aimed at developing genetic conservation strategies for threatened species and forest types. Some have also developed regeneration programs for conserved species and systems. The genetic conservation programs of Indonesia, Malaysia, the Philippines, and Thailand are presented and discussed in relation to their national programs for maintaining biological diversity. These nations, and others worldwide, also recognize the urgent need to establish, maintain, and monitor <i>in situ</i> conservation sites wherein entire ecosystems are kept in states of active interaction and evolution. They are unsure, however, of the appropriate scope of these undertakings and the resources and methodologies that may be needed to maintain effective conservation areas. Their interests, concerns, and information needs represent a challenging opportunity for the forest genetics research community.
CONSERVATION STRATEGY AND MANAGEMENT POLICY MUST CONSIDER BOTH LOCAL AND RANGEWIDE SCALES: PRESERVATION OF NATURAL GENETIC VARIATION IN THE WIDESPREAD TREE SPECIES SPANISH CEDAR.

Spanish Cedar, Cedrela odorata L., is a globally important timber species that, for more than 200 years, has been severely exploited throughout its natural range. Currently trees of good form are rare in the forest and natural populations have become substantially fragmented. Although there has been reasonable success in plantation forestry outwith the natural range of the species, the timber produced from such stands is usually inferior in quality to that obtained from autochthonous populations. It is important that the natural genetic resources of this widespread species are conserved and managed to provide a sustainable source of high-quality timber. For widespread species such as Spanish Cedar, genetic resource management strategies must consider variation at both local and rangewide scales. Genetic variation in 29 populations throughout the species’ range in Mesoamerica was analysed using chloroplast-specific neutral DNA markers. At the same time, a detailed analysis of genetic variation in ten Costa Rican populations was carried out using total genomic (AFLP) and chloroplast specific markers in tandem. At both rangewide and local scales a significant amount of intraspecific genetic variation was revealed, with a distinct spatial component. In particular, it was possible to identify evolutionarily significant units (ESUs), sufficient to conserve both historical and contemporary sources of variation, and management units, where populations with recent common history had become demographically isolated. These data indicate productive strategies for conservation of Spanish Cedar genetic resources at both international and national levels. The contrasting layers of population structure revealed by complementary local and widespread studies, emphasise the need for analysis at a variety of spatial scales when dealing with conservation of widespread species.
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INVENTORY OF THE GENETIC DIVERSITY IN BLACK POPLAR (POPULUS NIGRA L.) ACROSS WESTERN EUROPE. CONSEQUENCES FOR CONSERVATION AND NATURE DEVELOPMENT

Black poplar (Populus nigra L.) is a pioneer tree species of riparian ecosystems. Large areas of its natural habitat have been lost due to management of riverbanks, intensive grazing and wood cutting. During restoration of the natural borders of rivers, existing black poplar stands may act as source population for recolonization of floodplains. Therefore, it is important to know how much genetic variation in Black poplar is still present along different rivers. In addition, P. nigra genebanks may be used to generate source material for restoration. However, these genebank collections were originally set up for different purposes. Results will be presented of the EUROPOP project involving P. nigra populations along the borders of six European river systems and genebank collections in nine European countries, obtained with AFLP and microsatellite markers. The following topics will be addressed: the occurrence of clonal propagation in populations, and of duplications in collections; the occurrence of hybrid poplars and offspring of hybrid poplars; the amount of genetic variation within and among river systems; overlap between genebank collections; and selection of material for recolonisation.
POTENTIAL GENE FLOW BETWEEN THE ENDANGERED BLACK POPLAR (POPULUS NIGRA) AND CULTIVATED POPLARS. A CASE STUDY ALONG THE MEUSE ON THE DUTCH-BELGIAN BORDER.

The present study has been conducted to investigate (i) the potential of the existing black poplars along the Dutch-Belgian Meuse to act as a source population for recolonisation of the floodplains (ii) gene flow between the cultivated poplars and the native P. nigra and (iii) whether introgressed seedlings can colonise the riverbanks of the Dutch-Belgian Meuse. The study area is a nature conservation area of 3000 ha where the main objective is the restoration of river dynamics and the riparian ecosystem of the Dutch-Belgian Meuse over a distance of 50 kms. The genetic and phenological diversity of the remaining black poplar trees on the banks of the Dutch-Belgian Meuse was investigated by using AFLP and microsatellite markers and by observing the flower biology. Sixty-four seeds were collected from 2 open pollinated female black poplar trees and 33 seedlings that spontaneously colonised the river banks, were sampled. The genetic origin of the open pollinated progenies and of the Populus seedlings from the riverbanks was studied by using morphological characteristics, isozymes, the diagnostic locus win3, AFLP and microsatellite markers. The genetic diversity of the relicts was too small to act as a source for recolonisation along the Dutch-Belgian Meuse (only 4 different genotypes were found!). Although this is a limited study, the result show that a high level of potential introgression can occur. This is in contrast with other studies were low levels of introgression (0 to 5%) in black poplar were detected. Seedlings of the riverbanks were originating from spontaneous hybridization within P. x euramericana and between P. nigra and P. x euramericana. Introgressed seedlings seemed to be well adapted as they survived the river dynamics over several years. P. nigra cv. italica was a potential father for only one seedling. No seedlings of pure black poplar were found on the riverbanks. Based on these results and preliminary results of ongoing research on pollen competition between conspecific and heterospecific pollen, guidelines for minimising potential introgression and for the restoration of Black poplar along the Meuse are given.
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GENETIC DIVERSITY IN NATURAL POPULATIONS OF BLACK POPLAR IN UKRAINE

Abstract: Four Black poplar (Populus nigra L.) populations in Ukraine were studied. The populations are located along the Tysa and the Prut rivers. Both rivers belong to the Danube basin but they are separated by the Carpathians Mountains. Young leaf samples were taken from sexual reproductive and juvenile trees, which were chosen in each population using descriptors developed by the EUFORGEN Populus nigra Network. These trees were labelled and mapped using GPS system. The samples were analysed using microsatellites to assess the extent of clonal propagation, and the degree of genetic diversity and population differentiation. These data were then analysed and compared to the results obtained in a similar experimental set-up and using the same marker systems, of populations along seven European rivers during the EUROPOP Project. Scale of gene exchange within and genetic differentiation between rivers systems, considering the drainage as a metapopulation with several entities and migration events, were assessed. Level of hybridisation between Populus nigra and neighbouring P x euroamericana was estimated as well.
IN SITU GENETIC CONSERVATION OF A NATURALLY RESTRICTED AND COMMERCIAL WIDESPREAD SPECIES, PINUS RADIATA

PINUS RADIATA IS AN ENIGMATIC SPECIES, RESTRICTED IN EXTANT NATURAL RANGE, BUT GROWING IN COMMERCIAL PLANTATIONS WORLDWIDE THAT TOTAL OVER FOUR MILLION HECTARES. THE REMAINING NATIVE FOREST AND ITS ASSOCIATED GENE POOL ARE AFFECTED BY HISTORICAL AND CONTINUING LOSS OF HABITAT, FRAGMENTATION FROM CONTINUING DEVELOPMENT, INTRODUCED DISEASES AND INVASIVE PLANT SPECIES, DISRUPTION OF NATURAL DISTURBANCES SUCH AS FIRE, AND GENETIC CONTAMINATION FROM PLANTED AND NONLOCAL RADIATA PINES. ON A MORE SOCIAL LEVEL, THE SPECIES IS THREATENED BY VALUES THAT PLACE CONTRIVED RECREATION, HOUSING DEVELOPMENTS, AND NEW TRANSPORTATION CORRIDORS ABOVE SPECIES CONSERVATION. COMPLICATING THE INITIATION AND COORDINATION OF CONSERVATION ACTIVITIES ARE PRIVATE PROPERTY RIGHTS AND THE BINATIONAL (MEXICO/USA) DISTRIBUTION OF THE SPECIES. MORE POSITIVELY, RECENT ACTIVITIES BY UNIVERSITIES AND NONGOVERNMENTAL ORGANIZATIONS HAVE HELPED TO UNITE LOCAL CONSERVATION EFFORTS, CONDUCT ESSENTIAL SEED COLLECTIONS, AND INCREASE THE USE OF SCIENCE IN GENETIC CONSERVATION. EXAMPLES OF APPROPRIATE AND INAPPROPRIATE USE OF SCIENTIFIC INFORMATION IN GENETIC CONSERVATION OF NATIVE POPULATIONS OF RADIATA PINE WILL BE DISCUSSED.
In the last decade a new gene conservation network for forest trees has been formed in Hungary. This network outlined strategies for social broadleaves with economic importance (e.g. Quercus robur), rare or endangered species (e.g. Populus nigra) and noble hardwoods with economic importance (e.g. Pyrus, Sorbus). Within the network the tasks have been shared by governmental institutions, forest managers/owners, private nurseries and a professional organising committee which was formed by the Hungarian government. The gene conservation activities have been financed by a special governmental fund. Selection of plus trees or valuable populations has been carried out by managers/owners using guidelines of the organising committee. In specific cases molecular markers have to be used for selection to control taxonomic status or introgression. The most used marker is the Heinze’s specific markers for Populus nigra selection. This PCR-based analysis has been also accepted by the nurseries and the costs of the analysis can be included the price of cuttings/seedlings (less than 15% of the total price). Compared to other markers this PCR-marker has been proved as a fast, cheap, simply and safely using technique for both the official institute (designated authority, OMMI) and the forestry practice. RAPD markers were also used for characterisation of poplar clones in clone collections. These markers can be also analysed in laboratories with common equipment. Such markers should be widely used in forestry practice due to their reasonable financing level.
EFFECTS OF DOMESTICATION ON THE EVOLUTIONARY POTENTIAL OF EUROPEAN CHESTNUT (CASTANEA SATIVA MILL.)

Among widespread domesticated tree species, chestnut (Castanea sativa Mill.) can be taken as a model for studies aimed to combine conservation of evolutionary and adaptive potential, exploitation of breeding stocks and preservation of Mediterranean landscapes. This species is characterised by easy propagation through grafting, wide distribution and great economic importance due to its multiple uses like quality wood, food and fuel production. For these reasons man has influenced over many centuries its distribution range through propagation and transplanting of plant material, silvicultural practices and fragmentation of populations due to changes in land use. These factors, coupled with long-time massive pathogen attacks (Cryphonectria parasitica and Phytophthora sp.p.), have lead to a reduction of population size and depauperation of extant genetic stocks in many European countries. The present study is part of an EU funded project (CASCADE) aimed to characterise the variability of populations - wild and cultivated - from across Europe, with respect to molecular markers, adaptive traits, including drought tolerance and resistance to Phytophthora diseases with a view to its conservation and sustainable use during global warming. Here we report results related to a marker based analysis (isozymes and ISSR) of 27 Italian chestnut populations sampled according to their geographic distribution and domestication levels (fruit orchard, coppice, naturalised). Within and between population genetic variation, gene flow and linkage disequilibrium was estimated. The preliminary results indicate that in spite of the large human handling and movement of genetic material occurred in the past, a significant genetic differentiation among the examined populations was detected according to the geographic location of the sampling sites. Marked differences were observed in the Fis, Fit, Fst and Nm values within and between populations as well as domestication levels. Furthermore hints of possible differences among domestication levels were found in the amount of randomly-generated linkage disequilibrium. As expected, orchards showed large mean correlation of alleles among loci, eventually due to the nature of the genetic material used for the plantation and the management practices adopted. Surprisingly, significant differences were also found between chestnut coppices and naturalized stands (or ex-coppices), the former showing larger amount of disequilibria over the loci analyzed. Given the wide spectrum of different environmental conditions and geographic regions of the sampled stands, we interpreted the observed differences as possibly related to the past silvicultural practices. In coppices, the large number of trees stemming from the same stump/genotype (ramets) are expected to increase correlation among loci. This reduces the outcrossing rate, the overall genetic variance, the theoretical effective population size, and is expected to increase the inbreeding depression in the long run. If these results will be confirmed in a larger number of populations spanning the entire range of chestnut distribution, more appropriate criteria for long term conservation could be derived.
THE DOMESTICATION OF THE CULTIVATED CHERRY TREES (PRUNUS AVIUM) : COMPARISON BETWEEN GENETIC DIVERSITY OF WILD AND CULTIVATED CHERRY TREES.

One of the main events which modelled the current genetic diversity of cultivated species is the human selection through domestication and further genetic improvement. The knowledge of these processes is essential to preserve rationally the germplasm of these cultivated species. Prunus avium species includes wild cherry trees, used for their wood, and sweet cherry trees, cultivated for their fruits. Sweet cherry trees (cultivated compartment) are issued from the wild cherry trees (wild compartment) through domestication and selection processes. Before this domestication, glaciations events had modelled the genetic diversity of the wild compartment. P. avium is an allogam species and gene flow between wild and cultivated groups can occur. The study of genetic diversity in the whole P. avium species can allow to understand the evolutionary history of each compartment of this species, and especially to clarify the initial event of domestication and the importance of the gene flow having occurred between the 2 compartments. The objective of this work is to study the genetic diversity of the domesticated and the wild compartments of P. avium in order (i) to identify the patterns of recolonisation after the last glaciation, (ii) to precise the centre of domestication and (iii) to evaluate the occurrence of gene flow between the 2 compartments and the level of the genetic diversity in these 2 groups. The plant material is composed of 735 genotypes of cherry trees and wild cherry trees coming from 12 different European and Asian countries. We used AFLP and microsatellites markers to analyse genetic diversity. Results obtained from genetic diversity analysis in P. avium species led to three main conclusions. First, the analysis of genetic diversity in wild cherry trees allowed us to contribute to a better knowledge of recolonisation patterns of wild cherry trees. The Romanian and Georgian wild cherry trees appeared to be very differentiated from those of central and west Europe. The Georgian wild cherry trees showed the largest genetic diversity. This suggest that this area could have been a main glacial refugium. Second, a high proximity was observed between the whole cherry trees analysed and wild cherry trees belonging to western part of Europe. This result could indicate either the existence of a gene flow between the two compartments, or the existence of a domestication centre in this part of Europe. The presence of region-specific microsatellite alleles shared by cultivated and wild cherry could result from the occurrence of gene flow between the two compartments. However, it is difficult to prove the existence of a global gene flow because the differentiation between wild cherry populations is low. Third, the genetic diversity observed in cultivated compartment is lower than in the wild one. This better understanding of the organisation of genetic diversity will be taken into account to constitute a core collection of P. avium relevant genotypes to be conserved.
GENETIC DIVERSITY IN LARGE OLD GROWTH AND IN SMALL FRAGMENTED OAK STANDS: IMPLICATIONS FOR MANAGEMENT AND CONSERVATION PRACTICE.

The paper presents a comparison between the level of genetic diversity in large old growth natural stands and in small isolated stands from fragmented landscape. Several European white oaks were selected as model species. The genetic diversity was measured by isozyme gene markers. The results indicate that the large natural stands possess in most cases higher levels of variation, but the small isolated stands from fragmented landscape still harbour significant amount of variation, especially the old ones. The results could be of use in the forest management and gene conservation practice.
CONSERVING AUTOCHTHONOUS GENES IN FLANDERS: CREATION OF FOREST REPRODUCTIVE MATERIAL BASED ON INVENTORIES AND ON MOLECULAR MARKER DATA.

The growth sites of autochthonous trees and shrubs in Flanders have drastically been reduced. Obvious reasons are the processes of intensive forest exploitation and deforestation in the past centuries and more recently the scale enlargements in land use. Less known is the threat imposed on autochthonous genetic resources by the import of seeds from native species but of foreign provenance. Forest reproductive material of economically less important woody plants is often grown from seeds that originate from Eastern and Southern Europe, where cheap labour is at hand. These plants may be less adapted to the Flemish growth conditions because of their remote origin. Spread of foreign genes can influence the genetic constitution of local autochthonous populations of the species. A first step towards the conservation and use of autochthonous trees and shrubs in Flanders is an inventory. It opens possibilities for in situ and ex situ conservation strategies. Many trees and shrubs are planted in Flanders for reasons of nature conservation and natural landscape design. For these purposes, the use of plants with local autochthonous origin is desirable. To create autochthonous plant material seeds are collected at sites where autochthonous gene sources are still present. At the same time the best scored autochthonous trees and shrubs in the inventory are vegetatively propagated. These plants will serve as basic material for autochthonous seed orchards. Application of molecular marker techniques can be of help in the evaluation of autochthony of populations and has implications on the conservation strategies. An example of a practical conservation strategy based on chloroplast analysis of indigenous oaks will be elaborated.
ABSTRACTS

POSTERS
Castanea sativa Mill. is a wide-spread broad-leave species in Europe. It is of special importance in the Mediterranean region, as well as in Asia Minor, and it has been extensively used for its fruit and timber production for centuries. Hence, it has been subjected to a domestication process. In the present paper two chestnut populations coming from the Greek island of Lesvos, which is located in the northeastern Aegean sea, were studied by means of isoenzyme electrophoresis. One of the populations studied was wild (a coppice stand), while the other represented a grafted orchard. The results from the isoenzyme analysis were compared with previously published results from Greek and Turkish populations, in terms of genetic variability and genetic distance of the populations. Discussion investigates the potential effects of population isolation and examines the genetic relationships of the Lesvos island populations with other chestnut populations, capitalizing on their position in between continental Europe and Asia Minor.
Gene flow is an important force maintaining genetic diversity in plant populations. Despite its important consequences for the genetic composition of tree populations, accurate estimates of gene flow realized through pollen and seed dispersal are relatively rare. We investigated patterns of pollen and seed dispersal in a natural stand of Scots pine (Pinus sylvestris L.) located in the Tuchola Forests, in Poland. The population under study consisted of 321 adult trees about 160 years old and covered an area about 4 ha in size. For pollen dispersal study seed were sampled from nine centrally located mother trees in 1999 (n=1031) and 2001 (n=890). Additionally in 1999, 2000 and 2001, seeds were collected from several seed traps located on the ground (n = 208, 741, 185, respectively). Multilocus genotypes at nine isoenzyme loci were determined for adult and filial populations. Patterns of pollen and seed dispersal were investigated using the neighborhood model, based on pollen and egg haplotypes inferred from collected seeds. We found that only a small, although significant, proportions of viable embryos resulted from self-fertilization (3% in 1999; 8% in 2001). However, nearly 90% of all fertilizations were due to pollen coming from males located outside of the neighborhood areas (i.e. more distant than 50 meters - the neighborhood radius), likely from outside of the nearest stands. Pollen dispersal within neighborhoods was restricted to the nearest neighbors, however, the diameter at breast height (DBH) and directionality (relative location) of potential males were also important in determining male mating success. The seed immigration from outside of the neighborhoods (radius 50 m) of seed traps accounted for 7%, 39% and 20% in three consecutive years. The main factor affecting seed dispersal patterns was the distance to potential maternal trees. Additionally, in year 2000 several size-related characters of individual females (like DBH; crown width, volume and outer surface) and directionality influenced significantly female reproductive success. The results obtained in this study might have important implications for the management of genetic diversity of forest tree populations and may be useful for future programs of tree breeding and conservation of forest genetic resources.
**POLLEN MOVEMENT IN FRAGMENTED POPULATIONS OF THE ANDEAN OAK QUERCUS HUMBOLDTII BONPL...**

Population subdivision may affect plant genetic diversity by inducing selfing, reducing the potentially reproductive individuals, and by limiting population connectedness. We analyzed the behavior of outcrossing rates, inbreeding and gene flow by pollen in the recently fragmented populations of the Andean oak Quercus humboldtii Bonpl. using microsatellite loci. Specific questions were: 1) if fragmentation has affected the mating system, is there significant selfing, high biparental inbreeding, and low number of pollen donors? 2) Is there pollen mediated gene flow isolation in the fragments? Finally, 3) Is there a negative correlation between the size of the fragment, selfing rates, bi-parental inbreeding, and gene flow? Five fragments were selected with 9 to 60 adult trees in each one. At each site, I sampled three adults and 30 seeds or seedlings from each. Offspring arrays were genotyped for four microsatellite loci using standard PCR techniques and alleles were scored using silver staining procedures. Overall, the four markers provided more than 40 alleles, and a paternity exclusion probability of 93%. Genotype data was analyzed using the software MLTR for the mating system parameters. Results indicate that significant selfing (3%-9%) is occurring at the population, and fragment level. Biparental inbreeding is significant (0.049, SD +? 0.018), and is reduced in the larger fragments. The effective number of pollen donors is low (Nep = 1.3), indicating that few adults are providing most of the pollen in this landscape. Gene flow analysis indicates that fragments are receiving 32% of pollen from the outside on average, and is negatively correlated the larger the fragment. Fragmentation appears to affect Q. humboldtii in promoting selfing, mating among relatives, and by limiting the number of pollen donors, despite gene flow by pollen is ample.
Direct gene flow assessment is becoming an important challenge for ecologists. In plants, gene flow has two components: pollen and seed, that are seldom studied simultaneously. We have assessed gene flow via pollen and seed in a Prunus mahaleb population, a gynodioecious treelet pollinated by insects and seed dispersed by frugivorous. This population located in SW Spain and consists of 190 adult trees, which more that 95% are genotyped, in a southeastern Spanish population. This rosaceus treelet is pollinated by insects and seed dispersed by frugivorous animals. Both seed and pollen movement was analyzed by genotyping embryos using 10 microsatellites markers. We collected seeds dispersed by frugivorous animals from seed traps and tried to identify their source tree. When we applied Maximum Likelihood (ML) parentage analysis the exclusionary power was too low and a single mother tree could be unambiguously identified in only a few cases. However, by genotyping the seed endocarp, a tissue of maternal origin, we unambiguously assigned a single mother for 82.1% of the seeds. Pollen movement was analyzed through ML paternity analysis of those seeds with identified mother, plus 94 additional seeds collected directly from the mother tree. This information was used to described the spatial patterns of seed and pollen gene movement in our population and to detect long distance gene flow. Dispersion and pollination success of individual trees was also measured and their relation to landscape characteristics and reproductive features studied.
EXPLORING REPRODUCTIVE PATTERNS IN SORBUS TORMINALIS: INTERPLANT VARIANCE IN STANDS OF DIFFERENT SIZE.

This study has investigated variation in phenology, fruit and seed production, genetic diversity and mating patterns in both a large and small seed stand of Sorbus torminalis from northern Switzerland. The large stand (N = 96) occurred in an essentially continuous forest habitat with S. torminalis extending outside the study area; while the small stand (N = 27) was isolated from other S. torminalis by at least 3.7 km. The species is insect pollinated, is believed to be self-incompatible and generally occurs in low densities. In both stands, phenological and fruit set data showed a high degree of interplant variance, with plants in more open areas generally having larger floral displays and producing a higher proportion of fruit. However, as a proportion of stand size, less trees within the smaller stand produced fruit and they also produced fewer seed per fruit. Genetic diversity, determined from five microsatellite loci, indicated that the large stand had a higher average allelic content than the smaller population (NA = 14.6 ± 2.7 S.E. and 6.4 ± 0.8 S.E., respectively). However, observed heterozygosities (Ho = 0.76 ± 0.06 S.E. and 0.77 ± 0.08 S.E.) were not significantly different between the two stands. In terms of mating patterns and structure, the microsatellite data revealed that: (1) although the species is primarily outcrossing, several plants were also capable of self-fertilisation; (2) mating patterns differed among plants with evidence for both near-neighbour mating, as well as pollen movement throughout a stand; and (3) the large stand had a higher percentage of external gene flow than the small stand (47.1% versus 8.3%).

In conclusion, this study has highlighted interplant variation with respect to several aspects of reproduction in S. torminalis. Furthermore, the comparison between a large and a small stand suggests the occurrence of Allee effects in the latter (i.e. less potential mates leading to the production of fewer fruit and seed).
DEVELOPMENT OF MATERNALLY INHERITED MARKERS FOR NORWAY SPRUCE (PICEA ABIES)

Due to their maternal mode of inheritance in coniferous species, mitochondrial (mt) markers can be regarded as ideal tools for evolutionary and population genetic structure studies. The aim of the present study was to identify DNA polymorphisms in the mitochondrial genome of Norway spruce (Picea abies). Newly identified sequences of the mt-genome of Picea abies were used for the development of an easily applicable PCR based marker system. In order to identify mitochondrial sequences we attempted to isolate pure mitochondria from different conifer tissues. Embryogenic suspension cultures of Norway spruce proved to be suitable for the purification of mitochondria, using saccharose gradient centrifugation steps according to standard techniques. The purity of mitochondrial DNA was verified by applying PCR techniques using specific primers for nuclear, chloroplast and mitochondrial DNA respectively. Out of a partial mitochondrial library, one hundred clones were sequenced and 25 primer pairs designed. Only 3 (12%) of the primer pairs produced polymorphic patterns (length variation) when used to amplify Norway spruce DNAs from different geographical origin within Austria. The rest of the primer pairs gave multiple bands, no amplification product or a single monomorphic fragment. Controlled crosses were used in order to prove the maternal inheritance of the three polymorphic loci. The three respective regions were sequenced in different individuals and exhibited several insertion/deletion as well as base substitution. These new, PCR detectable polymorphic mitochondrial markers will facilitate the study of the population genetic structure of the Norway spruce.
NEW INSIGHTS INTO POSTGLACIAL GENE FLOW OF ABIES ALBA USING ORGANELLE DNA MARKERS WITH CONTRASTING MODES OF INHERITANCE

The conifer Abies alba (Mill.) turned out to be an excellent model to analyse the maternal as well as the paternal aspect of range-wide distribution of genetic diversity. Two DNA markers with contrasting modes of inheritance were applied to 100 populations covering the entire range of silver fir in Europe. 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, the psbC cline was as wide as the whole range of the species. Our results strongly suggest that an exchange of genetic information between refugia by range-wide paternal introgression is possible in this wind-pollinated species.
SEED-MEDIATED GENE FLOW AND GENERATIONAL CHANGE IN GENETIC STRUCTURE WITHIN A NATIVE BRITISH OAK WOOD.

The peculiar seed dispersal dynamics of oaks (heavy local fall and occasional very long distance dispersal) have been invoked to explain the observations of significant structuring of genetic variation within oak forests on a fine spatial scale (<100 m) and the occurrence of large patches of oak forest (up to 100s km across) dominated by a single chloroplast DNA variant (‘footprint of colonisation’). The distance and frequency of seed-mediated gene flow is a central premise of these hypotheses. This study presents data on seed dispersal distances within a native British oak wood. The change in fine-scale genetic structure within the wood from acorn through seedling to adult tree cohort is described, and influences on generational change in genetic structure are discussed. In addition, the observed seed dispersal distances obtained for Quercus robur and Q. petraea are used in a simulation modelling approach to predict genetic structure resulting under different gene flow scenarios and compared to observed patterns at a population and regional scales (across the British Isles).
Genetic drift is considered one of the main evolutionary factors whose shaping force in determining the current genetic structure of forest tree species is normally assumed in theory but only in few cases has been proved. Small marginal populations of Austrocedrus chilensis (D.Don) Pic. Ser. et Bizzarri in the North Patagonian steppe constitute a proper system to study genetic drift processes since they have been probably subjected to genetic isolation during several hundred thousand years. In order to test this hypothesis, three marginal populations were selected and their genetic structures characterized by genotyping seed trees and their progeny at 12 isozyme marker loci in which 28 alleles were detected through a previous segregation genetic analysis. Genetic structures of other 14 populations distributed in the whole Argentine range of the species were considered as comparison reference. Relative high frequencies of usually rare alleles were found in these populations. Moreover, one of the populations has a relative frequent (0.107) exclusive allele not present in the other 16. These results could be seen as evidence of drift; however, different selection pressure and life histories might be the cause for such differential patterns. A new analysis was therefore proposed. An additional marginal population neighbor to one of the previous ones (located just 1.2 km southward) was included in the study. Surprisingly, a genetic distance bigger than any calculated between other populations of the species was obtained (d0 = 0.126). Furthermore, a great qualitative difference between their allelic structures could be detected. Population «A» has two relative frequent genes (Mdhl-2-72: 0.107 and G01-97: 0.375) not present in population «B», while a rather frequent allele in «B» (Got-50: 0.441) could not be found in «A». Since different life histories as well as differential selection processes should be in this case discarded due to their vicinity and environmental similitude, the found patterns have to be seen as an indicator of an accentuated genetic drift effect in combination with a restricted interpopulation gene flow. The strong unidirectional West-East wind very likely helped to avoid the genetic information exchange. To our knowledge this strong evidence of genetic drift constitutes one of the seldom, if ever, cases found in forest tree genetic population studies.
MEASURING GENETIC DIVERSITY IN MARITIME PINE TO IMPROVE CONSERVATION AND MANAGEMENT

Maritime pine is one of the most important conifer species in the south-western countries of Europe, particularly in Portugal, where it occupies the largest area of forest. The understanding of the genetic variability of this species is important for breeding, seed certification, evaluation of seed production areas, and for conservation purposes. Recently chloroplast and nuclear microsatellites were used to screen the genetic diversity of Pinus pinaster in Portugal. We are genotyping 60 orchard clones using total DNA extracted from needles. Other genotypes collected from regions surrounding the orchard were also included. Both chloroplast and nuclear microsatellites are being used and the frequencies of the alleles and the haplotypes will be assessed on the basis of band size by using standard markers as described by for cpSSR and for nuclear SSR.
The pedunculate oak (Quercus robur L.) populations indigenous to the Middle Near Volga region of Russia represent a valuable gene resource. These oak forests grow on the northeastern boundary of pedunculate oak's natural range in the Europe and have existed for a very long time. Oaks in this region are characterized by some peculiarities in intraspecific variability and genetic structure. Three phenological forms have been distinguished by differences in timing of autumn coloration and leaf fall. Progeny tests of the different forms show differences in early growth rate and demonstrate higher susceptibility to leaf damage. This study assessed intraspecific variability of trunk, branch, crown, bark, and leaf morphology. On the base of the study of leaf morphological traits spatial distribution two groups of populations were distinguished: Privolzhskaja forest and Zavolzhskaja forest-steppe. The latter group is comprised of two subgroup populations growing in a forest-steppe zone of region: Mokshansko-Surskaja (highland) and Zavolzhskaja (high plain). They are characterized by particular differences in morphological and adaptive traits. Isoenzyme analysis of 14 isoenzyme systems controlled by 21 loci, revealed high genetic diversity of oak populations in this region. The mean percentage of polymorphic loci (50-68.8%), by 95% criterion, and the average number of alleles per loci (1.8-3.1) was determined. Effective number of alleles nes – 1,43. The observed heterozygosity (0.131 – 0.222) was always lower than expected (0.122 – 0.251) and the deficiency of heterozygots was concluded. Calculating of Nei's (1978) genetic distance (DN - 0.0016 – 0.0065) coefficients and the genetic distance (D) after Gregorius (1974) indicates the low genetic differentiation between the oak populations in the whole area in the European Russia. The populations share a common gene pool with intensive gene exchange between populations (Nem = 9.9). No clear clinal patterns of association of isozyme variation with the location of population in current study were observed. As a whole genetic diversity of oak populations in the European part of Russia are in the middle between the same characters in Finland and populations from Central and Western Europe. The analysis by means of RAPD markers just confirms the revealed patterns. The concentration of variability within a population as opposed to between populations suggests that allocation of three or four to five smaller genetic reserves in borders of groups of populations over a variety of sites would adequately conserve a significant portion of the genetic variability. All silviculture measures should consist in creation of tolerant, multi-storeyed mixed stands of oak with attendant species which forms protective “fur coat” and promotion for oak. We had developed a special system of recover of declined oak stands based both on natural and artificial regeneration.
Gene flow is known to be a crucial factor for the success of breeding and conservation programs. In seed orchards, pollen contamination (PC, i.e., pollen-mediated immigration) may reduce genetic gain and cause maladaptation of the produced seedlots. It is difficult to assess the effectiveness of techniques developed to reduce PC because the precision of its quantification depends on the variability of the available genetic markers. Microsatellites, or Simple Sequence Repeats (SSRs) have the potential to provide sufficient genetic resolution for studies of gene flow by parentage analysis, and therefore, for reliable estimation of PC.

The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) and Oregon State University launched a project aimed at developing microsatellite markers for studying PC in Douglas-fir seed orchards. In this study, clones containing large, low-copy inserts were selected from 5 microsatellite-enriched genomic libraries. 517 such inserts were sequenced and screened for the presence of either dinucleotide [(AC)n, (AG)n], or trinucleotide [(AAT)n, (ATC)n] SSRs. 385 SSR-containing clones were found, and primers were designed for 110 of these SSRs. By testing these markers on agarose gels, 34 promising SSRs were identified, and further characterized using fluorescent-dye-labeled primers and capillary electrophoresis. 21 of these markers produced the desired single-locus polymorphic pattern when used to amplify embryo and megagametophyte DNA from Douglas-fir seeds. The other primer pairs gave either multiple bands (indicative of two or more loci) or a single monomorphic band. Codominant Mendelian inheritance was observed for all 21 single-locus, polymorphic markers. The alleles of 15 markers were surveyed in an average of 79 (60-95) trees. The average number of alleles per locus was 31, ranging from 17 to 42. The observed heterozygosity was 0.88 (0.70-0.94). These SSR markers should provide a powerful tool for gene flow studies in Douglas-fir.
PHENOTYPIC AND GENOTYPIC VARIATION AMONG SALIX ALBA - S. X RUBENS - S. FRAGILIS IN FLANDERS

The individuals of the S. alba - S. x rubens - S. fragilis complex are morphologically difficult to identify. Frequent hybridization and introgression between the species of the complex fade the boundaries between the hybrid S. x rubens and its parents S. fragilis and S. alba. A combined morphological and molecular-genomic study has been performed on about 100 individuals in order to get insight into the classification of the taxa and to improve our knowledge of the phenotypic and genotypic variation within the willow complex. Different willow varieties and hybrids belonging to the complex were sampled along the rivers Schelde, Maas and IJzer. Mature leaves, twigs and flowers were collected from the lower part of the crown in order to identify the specimens according to Meikle (1984). AFLP analysis was used to characterize the samples at the molecular level. The morphological analysis revealed that several characters of twigs, leaves, petioles, stipules and flowers can discriminate between S. alba and S. fragilis and between some of their varieties. S. x rubens displayed for some of these characters an intermediate position between both parents. AFLP-fingerprinting of the investigated trees resulted in 236 informative polymorphic bands. All individuals showed a unique banding pattern. No species-specific markers were found, although 11 markers displayed significant frequency differences. Multivariate analyses of both the morphological and the genetical data revealed two main groups with 1) S. alba varieties and S. x rubens and 2) S. fragilis varieties and S. x rubens var. basfordiana. The fact that the S. x rubens individuals do not cluster together at an intermediate position, may be explained by matroclinal inheritance (Krstinic, 1966). In this case S. alba would be the female parent of S. x rubens, while S. fragilis would be the female parent of S. x rubens var. basfordiana. Furthermore, if S. x rubens specimens tend to backcross with S. alba and S. x rubens var. basfordiana with S. fragilis, they become indistinguishable from the mother line. A parallel flowering period of S. alba and S. x rubens and of S. fragilis and S. x rubens var. basfordiana, together with similar frequencies of occurrence of the 11 diagnostic markers support this theory. Meikle (1984) distinguished different varieties of the two parental species. Furthermore, he supposed that the varieties S. fragilis var. russelliana and var. furcata are clonal. However, in our study, the results of both the morphological and molecular analyses revealed that it is very difficult to recognise S. alba and S. fragilis varieties in Flanders. Furthermore, the genetic variation within S. fragilis var. russelliana and var. furcata is too wide to represent clones. The most acceptable theory about the origin of these varieties is that they developed several times independently from different individuals of the type species S. fragilis var. fragilis.
Germplasm of Japanese and Chinese chestnut was introduced in South Europe since first decades of the twenty century to be used in areas in which chestnut was damaged by the ink disease, caused by Phytophthora sp. After 1940, several hybridization programmes between European chestnut Castanea sativa Miller with Japanese chestnut, C. crenata, or Chinese chestnut, C. mollissima were initiated in Spain, France, Portugal and Switzerland. The objective was to get individuals resistant to Phytophthora and to Chryphonectria parasitica for wood or fruit production. Since them clonal plantations of hybrids were expanded. According with the results of plantations hybrids seem to be less tolerant to drought and to frosts than the European chestnut. The introgression of the Asiatic germplasm in the European chestnut wild populations has a high probability to occur due to the broad period of flowering overlapping. In 1989 was initiated a programme at CIFA Lourizán to identify a collection of 177 hybrid clones. Another objective was the selection of clones for forest use. Morphological traits and isozyme loci useful for species and hybrids identification are summarised. Records of growing, bud set, flushing and flowering made in clonal tests of hybrids with C. sativa local sources as controls are used to compare adaptive potential of hybrids and C. sativa and flowering overlapping.
LOCAL DYNAMIC OF INTERSPECIFIC HYBRIDIZATION BETWEEN TWO SOUTHERN BEECHES (NOTHOFAGUS SPP.)

Natural hybridization constitutes one of the main sources of intra-generic genetic variation in forest tree species. The lack of species-specific genetic markers has been an important obstacle to study this evolutionary process and to understand its general and local dynamic. Nothofagus nervosa and Nothofagus obliqua are two sympatric southern beeches, native to southern South American temperate forests that hybridize naturally and for which two independent specific allozyme marker loci have been determined allowing the identification of F1 and backcrosses-hybrids. The main hybridization direction with N. nervosa acting as mother tree could be also verified. Although the general process has been already studied many aspects of local temporal and spatial dynamics of the inter specific hybridization are still unknown. Three adult trees of N. nervosa within N. obliqua forest, almost totally isolated from other co specific trees and with different sympatry degree were selected. Genotypes of them and of the up to 100 m surrounding saplings (84, 129, 64) were determined. In two of the mother trees, seeds from two and three consecutive harvest years were also genetically identified. Significative differences resulted from the comparison among the genotypic structures of the three groups of saplings analyzed, particular in the proportions of the respective F1-hybrids (8.33, 1.55, 0.00). Also the proportion of hybrid seeds varied between both mother trees analyzed and among harvest years within each tree (e.g: 77-22%). The proportion of hybrids detected in the seed of all harvest years was bigger than that of the hybrid saplings from the corresponding mother tree. This difference is bigger in the case of F1-hybrids (up to ten times) than for the backcrosses-hybrids. These results indicates that the realization of natural hybridization at individual level between these Nothofagus spp. depends greatly on the particular site conditions of the respective N. nervosa mother tree (e.g. sympatry degree) and the climatic conditions of the corresponding year. Natural selection against F1-hybrids is much more greater than against backcrosses-hybrids confirming the general prediction previously postulated in the proposed hybridization model.
AN INVESTIGATION INTO THE TAXONOMIC STATUS OF IRISH OAK

Oak samples were taken from woodlands across Ireland and assessed using morphological and molecular analyses. The morphological analysis consisted of an investigation of a number of leaf characters using multivariate techniques. The molecular analysis consisted of cpDNA, SSR and AFLP analysis. The morphological data revealed a grouping of the individuals into the species, Quercus robur and Q. petraea, although a number of intermediate individuals also existed. However, the molecular data did not show such a distinction and very few differences were detected. In this paper a discussion is presented based on these findings.
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PCR-RFLP ANALYSIS OF CHLOROPLAST DNAs IN SOME ABIES SPECIES

Genetic relationship between some species of the Mediterranean, North American and Asian firs (Abies sp.) was studied using PCR-RFLP analysis of chloroplast DNA (cpDNA). One gene region (rbcL1-rbcL2) and four intergenic spacer regions (trnV-H, trnC-D, trnL-V, psbC-trnS) of the chloroplast genome were analyzed. Based on twelve digestions of the respective PCR products the dendrogram was constructed illustrating genetic differentiation between the Asian species A.homolepis, A.holophylla, A.veitchii, A.koreana, A.sachalinensis and A.fargesii and the Mediterranean species A.alba, A.nordmanniana, A.cephalonica, A.cilicica, A.pinsapo, A.numidica and A.nebrodensis including their hybrid forms A.borisii-regis, A.equi-trojani and A.bornmuelleriana. The North American species A.lasiocarpa was clustered together with A.fargesii showing higher affinity towards the Asian firs than to the North American ones. The same was true of the Asian species A.mariesii which occupied common position with the North American species A.procera and A.magnifica. Occupying relatively independent position on a dendrogram the North American species A.concolor, A.grandis and A.amabilis deviated conspicuously from the rest of North American species. In general, there was observed a close relationship between position of individual species on a dendrogram, their taxonomic status and crossability. In particular it concerned the hybrid forms A.borisii-regis, A.equi-trojani and A.bornmuelleriana occupying intermediate positions between their respective parents A.alba, A.nordmanniana and A.cephalonica. Based on PCR-RFLP analysis of cpDNA, the Mediterranean firs seem to be genetically closely related deviating profoundly from the Asian and North American species. The two last mentioned groups of firs exhibit some degree of genetic relatedness. The only difference is that Asian firs are much more closely related than those of North America which seem to be genetically rather heterogeneous.
OAKFLOW is a EU supported project gathering 14 different labs from 11 countries. The project aims at estimating the amount of intra- and interspecific (hybridisation) gene flow in European oaks (Q. petraea and Q. robur mainly but also other Mediterranean white oaks) by using genetic fingerprints and applying parentage analysis. Natural gene flow due to pollen and seed dispersal is being investigated in 13 ISPs in Europe. An ISP is an intensive studied plots covering from 5 to 20 hectares where all trees are genotyped and mapped. In most cases the ISP were mixed and composed at least two interfertile oak species. The consequences of gene flow at the genetic but also ecological level are addressed. Genetic maps are being constructed for the different species and QTLs for various morphological and ecophysiological traits involved in species differences are detected. Genomic regions affected by hybridisation are identified and mapped along linkage groups. Fitness related traits will be compared between hybrids and the parental species. These comparisons are conducted on juvenile and adult material, in natural stands and in nurseries with controlled hybrid families. Finally, impacts of gene flow on management rules will be addressed by considering different real cases in strong cooperation with end-users associated to the project.
Hybridization is a common process in oaks although few cases have been described in Mexico where more than 150 species have been diversified. Quercus dysophilla has been nominated as the putative hybrid between Q. crassifolia and Q. crassipes (Fagaceae: Quercus: Subgen Erythrobalanus). This putative hybrid is geographically distributed in the overlapping region of these two parental species at the Eje Neovolcánico in Central Mexico. We examined if Quercus dysophylla is a hybrid plant formed by the Q. crassifolia x Q. crassipes complex in the hybrid zones located at the Eje Neovolcánico in Central Mexico, and to determine if the nearness of the hybrid plant to the allopatric site of a parental species, increases its richness of gall forming insects and morphological similarity with its parental. In total, we measured 17 morphological traits of 5700 leaves in 190 trees: Q. crassifolia (n=70), Q. crassipes (n=70) and the hybrid, Q. dysophilla (n=50). Q. crassifolia differed significantly from Q. crassipes in all the characters examined. Q. dysophylla had intermediate leaf characters between Q. crassifolia and Q. crassipes, assessing the hybridization hypothesis. Discriminate function analysis (DFA) for size and shape of the leaf, showed that the leaf morphology of the hybrids resulted intermediate between parental species. In average, for all studied sites, the 43.33% of the total variation in size and shape was attributable to shape alone. Principal component analysis (PCA) showed that the hybrid can be separated from the parents group in all sites, and in average for all sites the two axes jointly accounted of 96.89% of the total variation. In order to corroborate these results we used molecular markers (RAPD's), which supported the hybridization hypothesis. Also, we observed that the nearest the hybrid plant was from an allopatric site of a parental species, it showed more similarity (in leaves morphological characters as well as in the richness of gall forming insects) with its parental and vice versa.
The main objective of this EU-research project (DYNAMO) is to use and further develop molecular tools as screening methods to determine the genetic identity of polyploid and introgressed populations. The use of different complementary approaches on the same carefully sampled material will enable accurate appraisal of the usefulness of the different techniques in: (i) genotyping polyploid individuals; (ii) revealing hybrid identity, and; (iii) estimating the extent of introgression in natural populations, all of which are very important issues in conservation genetics. The molecular tools that are included as screening methods in this study are: 1) a selection of AFLPs (Amplified Fragment Length Polymorphisms) and RAPDs (Random Amplified Polymorphic DNAs) (partner 01) 2) enzyme consensus primers of conserved sequences (partner 01) 3) nuclear SSRs (Simple Sequence Repeats) (partner 02) 4) cpDNA (chloroplast) and mtDNA (mitochondrial) sequence analysis (partner 03) 5) cp SSRs (chloroplast Simple Sequence Repeats) (partner 04) Different molecular tools have been tested on this material coming from full-sib crosses as well as from natural and planted stands along different European rivers. The use of co-dominant markers is very promising to describe patterns of hybridisation at a local scale, while both the dominant markers and haplotypic markers are promising to describe patterns at a larger regional scale.
GENETIC CHARACTERISATION OF QUERCUS SUBER L. FOR BIODIVERSITY CONSERVATION AND FUTURE BREEDING

Cork oak (Quercus suber L.) is an important forest species both from ecological and economical points of view in the Mediterranean basin. In the recent years it has gained an increasing scientific interest, thanks to the divulgence policy and research collaborations promoted by Euforgen. Within the Concerted Action Program (FAIR 1 CT 95-0202) on the «Evaluation of genetic resources of cork oak for appropriate use in breeding and gene conservation strategies», we established field trials with germplasm representative of the entire area of origin of the species, as a first attempt to estimate the available cork oak genepool for adaptive traits and quality end uses (silviculture regime and cork quality and quantity production). In order to improve the available knowledge on the genetic structure of cork oak, so far limited to a few studies on storage proteins, allozymes and IGS maps, we tested the usefulness of rDNA Internal Transcribed Spacer (ITS), a well known neutral molecular marker. Individual samples from the main distribution areas of the species (Italy, France, Spain, Portugal, Morocco, Algeria and Tunisia) have been analysed by means of PCR-amplified ITS nucleotide sequences. A more exhaustive investigation has been conducted on Italian populations, with a special regard to Latium (Central Italy). The sequence homology data show that all the tested provenance can be grouped in two distinct clades: one set gathered together the Franco-Iberian populations and the other comprises the populations from North Africa and Italy. The only exception is represented by the Apulian population, the most Eastern site, which stands alone confirming a relevant genetic diversity as already reported in Bellarosa et al. (1997). Our data are in agreement with previous results obtained by Toumi and Lumaret (1998) and Jiménez et al., (2000) on the basis of the detected isoenzyme polymorphism. For what concerns populations from Latium, higher affinities are shared by neighbouring stands, even if the low level of polymorphism detected by the ITS sequences might be not fully indicative of the real genetic relationships between the samples. More appropriate, high potential molecular markers are therefore needed to characterise closely related populations of cork oak. This kind of investigation appears of particular urgency after the publication of the "Commission Regulation (EC) No 1602/2002 of 9 September 2002 laying down detailed rules for the application of Council Directive 1999/105/EC, as regards the authorisation of a Member State to prohibit the marketing of specified forest reproductive material to the end-user" which, in turns, might threaten the conservation of forest biodiversity. Bibliography: R. Bellarosa, B. Schirone (1997) IPGRI, Euforgen, Med. Oaks Network, 23-31 P. Jiménez, R. Alía, L. Gil (2000) IPGRI, Euforgen, Med. Oaks Network, 15-20 L. Toumi, R. Lumaret (1998) Theor Appl. Genet. 97, 647-656
EFFECTS OF NITROGEN STRESS ON ADAPTIVE GENETIC VARIATION IN ACER PLATANOIDES AND BETULA PENDULA

Seedlings from one Swedish and one Norwegian population of Acer platanoides L. (Norway maple) and Betula pendula Roth. (silver birch) were cultivated for one growing season in both a controlled free access (non-stressful) and a low nutrient (stressful) condition. We analysed whether the nutrient treatments had an effect on genetic variation in growth and phenology traits, and investigated the population and family rank changes between environments. The nutrient effect was strong indicating high phenotypic plasticity in both species. Populations of Norway maple differed little whereas the family variation was high between and within treatments. Treatment rank changes were also higher at the family than at the population level. In silver birch, populations differed for half of the traits between and within both treatments. Except the high variation among non-stressed Norwegian families, family differentiation was generally low and the treatment interaction at the population and family levels were moderate. The relation between the species life-history traits and the partitioning of genetic variation within and between populations were unclear. According to our knowledge, this is the first study of reaction norm variation in fitness-related traits in temperate deciduous tree species.
CYTOGENETIC MECHANISMS OF ADAPTATION IN SPECIES - INTRODUCENTS.

Introduction is one of a way for genetic resources conservation of valuable species including forest trees. Only those species and forms could be successfully introduced in a new habitat, which have wide norm reaction of general tolerance to the changed environmental conditions (some of them could be considered as extreme for them). This feature is often accompanied with genome changes, which can be recorded using light microscopy. We analyzed meiosis and mitosis in Picea glauca (Moench.) Voss. = P. canadensis B. S. P., Tsuga canadensis (L.) Carr., Pseudotsuga menziesii (Mirb.) Franco., introduced into Voronezh region from Canada, Cotinus coggygria (Scop.) – from Caucasus, and Mediterranean, and Ulmus pumila (L.), from Middle Asia. Two types of cytogenetic mechanisms of adaptation can be discriminated: general and specific. The mixoploidy was a general type of adaptation, which regulates the ploidy level depending on alterations of environmental conditions. This type was prevailing in Ulmus pumila (L.) and it ensured partial fertility to mixoploid plants. A species-specific mechanism of adaptation revealed in Picea glauca (Moench.) Voss. = P. canadensis B. S. P. was the appearance of additional B-chromosomes, which regulates the amount of heterochromatin. The different specific mechanism of adaptation have been revealed by us in other species. The occurrence of B-chromosomes was shown to be the main character which should be taken into consideration in selection of adaptable forms in the species Picea glauca. The B chromosome-like structures were firstly discovered in Tsuga canadensis, although mitosis as a whole was regular. However, we found various types of meiotic disturbances in this species which resulted in complete sterility of plants having normal habitus. In Canada Tsuga canadensis become a seed-bearing to 60 years old. Investigated by our trees are 30 years old also. Therefore we can hope that meiosis in Tsuga canadensis may be stabilised to 60 years. Various level of elimination of chromatin was observed in some fertile forms of Cotinus coggygria. Owing to such deminution of chromatin in microsporocytes and, possibly, macrosporocytes, the other sporocytes can develop normally and produce fertile seeds. Normal fertility of Pseudotsuga menziesii trees was directly associated with meiotic regularity. Homeostasis in fertility trees of Pseudotsuga menziesii was provided by regulation of nucleoli activity. In such trees nuclei with 6 nucleoli were prevailing in comparison with the seeds collected from trees are growing in Canada. The knowledge of cytogenetic mechanisms of adaptation of species-introducents will make a valuable contribution in selection and conservation of forms and species better suited to a new habitat.
COMPARISON OF ISOZYME AND RAPD MARKERS FOR REVEALING GENETIC VARIATION IN WALNUT SPECIES (JUGLANS REGIA L.)

Isozymes and RAPDs each have advantages and disadvantages as markers for assessing genetic variation. However, relatively little effort has been devoted to applying both types of markers to the same set of individuals. Such a study would provide experimental data to verify the relative merits of the different methods and would indicate whether measures of variability obtained by one method are similar to those obtained by the other one. Hence, the objective of this research was to compare these markers for assessing genetic variation in walnut species (Juglans regia L.). A total of 7 loci from 4 enzyme systems and 14 RAPD markers were analysed in 56 different walnut genotypes. Metric and non-metric similarity coefficients (Jaccard’s, simple matching, etc) of the samples were computed. Cluster analysis, principal component analysis were conducted to reveal the relationships of the samples at DNA and isozyme level. Because data on ecogeographical variables were available, we also employed other statistical model to find out the most important ecogeographical variable(s) that influence the genetic variation observed with both molecular markers.
GEOGRAPHIC VARIATION IN WALNUT (J. REGIA L.) IN THE NORTHWEST OF SPAIN

A walnut (J. regia L.) plus tree prospection was made in the Northwest of Spain (Galicia) in 1997. Forty-three plus trees were selected during that year. In spring 1998, a progeny test was planted at two different sites. Several data were registered: survival, growth (total height and root collar diameter), phenology (bud burst and leaf fall) and late spring frost and blight damages. The relation between cited variables and geographic and climatic variables of the origin place is studied. Results of data analyses show a clinal variation NW-SE, resulted of an adaptation to ecological conditions of seed origin. So, progenies from the Northwest of the prospecting area, nearby the sea, are more vigorous, leaf fall later, and suffer less frost and blight damages than those from the Southeast.
IMPROVING FRAXINUS (ASH) PRODUCTIVITY FOR EUROPEAN NEEDS BY TESTING, PROPAGATION AND PROMOTION OF IMPROVED GENETIC RESOURCES.

This EC project has 14 partners in eight countries, details are available at http://www.teagasc.ie/advisory/forestry/rap/index.htm. The genetic diversity and growth potential of European ash is being made by taking measurements and growth data from existing provenance trials and by using microsatellite analysis. First results have shown significant differences between provenances for height and circumference data and genotypic heritability varied between 0.32* and 0.76*** on two sites examined. The coefficient of phenotypic variation decreased with time; it ranged from 15-19% for data collected in 1990 to 5-6% for data collected in 2000. The analysis of data on stem form and crown form showed very highly significant differences among provenances and heritability was characterised as medium to very low (from 0.68 **** to 0.26 NS). Provenance effects were also very highly significant for frost damage at two sites and genotypic heritability was good (from 0.60 *** to 0.72***). The genotypic heritabilities and gains were computed after a multi site analysis of all characters measured. This showed that provenance effect was highly significant for height growth but not for height increment and stem girth. Height heritabilites were 0.78*** from 1990 growth data, decreasing to 0.53** for 2000 data. Provenance effect was at least, significant for crown and stem form giving genotypic heritabilities from 0.44* to 0.77***. Five nuclear microsatellite markers were used to analyse genetic diversity, gene flow and hybridisation. The first estimates of genetic diversity showed a high level of diversity within populations. More detailed analyses showed a deficiency in heterogyosity in some populations. Seed and gene flow was also examined: see Abstract by M. E. Morand et al. . Plus trees were selected among the best provenances and populations, grafted and conserved as well as being micropropagated. Viable shoot cultures were obtained from 20 selected trees and viable plants from five selections of mature trees are in clonal field tests. A study on the adoption of new information, materials, and technology by the end users of ash is in progress in relation to the market situation and industry needs.
SENSITIVITY TO ROOT HYPOXIA AND MORPHOLOGICAL ADAPTATIONS IN QUERCUS ROBUR: INTRASPECIFIC VARIABILITY AND DETECTION OF QTLS

Quercus robur L. (pedunculate oak) and Q petraea (Matt.) Liebl. (sessile oak) are two sympatric broad-leaved forest tree species of western Europe, that generally occupy different but proximal ecological niches. Although morphological and ecophysiological differences are found between them (Dupouey and Badeau, 1993; Bréda et al., 1993; Ponton et al., 2001) the two species exhibit low genetic differentiation (Bodénès et al. 1997). Nevertheless, several ecophysiological and morphological traits differentiate them very clearly. Among those may be traits related to the tolerance to water-logging, Q robur growing in bottomlands periodically subjected to flooding and Q petraea on top of hills with deep and well drained soils. A first step to this question was to assess the degree of variability in such traits within the species Q robur. A QTL approach was used basing on 182 individuals from of a full-sib family propagated through rooted cuttings. Two copies of each genotype were used and submitted to either seven or twelve weeks of complete flooding. The trees were very tolerant even to this severe stress a no death or wilting occurred even after twelve weeks of the treatment. At the end of the stress period trees were harvested and the flooding-induced changes in anatomy and structure were assessed: number of lenticels and development of adventitious roots. Amount of dead roots was also assessed as an indicator of the plants' sensitivity to root hypoxia. Significant QTLs were detected for the number of lenticels and of adventitious roots, located on the same linkage group. Two further significant QTLs were detected for the quantity and fraction of dead roots. This suggests that the within family variability of sensitivity (dead roots) and tolerance (lenticels and adventitious roots) to hypoxia tolerance of pedunculate oak has a genetic basis. Further steps will include QTL analysis of these traits in an interspecific cross (robur * petraea) and comparisons of the physiological basis of hypoxia tolerance in oaks species. Bodénès C., Joandet S., Laigret F. & Kremer A. (1997) Heredity 78, 433-444. Bréda N., Granier A., Dreyer E. & Cochard H. (1993) Annales des Sciences Forestieres 50, 571-582. Dupouey J.-L. & Badeau V. (1993) Annales des Sciences Forestières 50, 35-40. Ponton S., Dupouey J.-L., Breda N., Feuillat F., Bodénès C. & Dreyer E. (2001) Plant, Cell and Environment 24, 861-868.
The relation between the performance in adaptive traits and the genetic differentiation and variability patterns was examined in fir populations from Greece. In particular, nine fir populations growing in a provenance-test plantation located in central Greece, within the distribution range of the species, were used. The places of origin of the populations cover the range of the fir species distribution in Greece. Four of them represent places where the distribution is continuous and the other five occur in sites of an island-type distribution. The growth and survival rates were measured, and compared to the results taken by analyzing isoenzyme markers, as well as previously published results of morphological traits and terpene markers. Similar patterns of genetic differentiation were obtained by the analysis of isoenzyme markers, terpenes and morphological traits. The results of the cluster analysis indicated that the genetic differentiation observed is in concordance with the geographical distribution of the studied populations. Genetic diversity in isoenzyme markers as well as growth and survival rates were lower in isolated populations originating from marginal areas. Implications for conservation and breeding are discussed.
In France, a provenance test network of sessile oak has been planted from 1989 to 1996. The network comprises four sister plantations located in the western part of France, the Centre, Burgundy and the North-East. We test 115 populations of sessile oak (Quercus petraea) and 15 pedunculate oak (Quercus robur) from all the natural ranges of the 2 species, 75 are French. Each population in each plantation is represented 240 to 1440 individuals. The network has been evaluated for different characters like phenology (date of bud burst, leave retention), growth (total height, DBH) and form (forking, straightness,…). The Qst vary from 7% to 85%. The phenological characters show clinal patterns with the highest structuration, the growth characters have intermediate structuration and the architectural characters have the lowest structuration.
GEOGRAPHIC VARIABILITY ON FLUSHING OF IBERIAN WILD CHESTNUT POPULATIONS

Bud break is one of the most interesting traits related with geographic variability of forest tree species in Temperate regions. After winter rest, winter buds initiate flushing when a determinate sum of temperature is reached. Some species need also to reach a determinate number of cold hours before the heat period accumulation. In regions with cold temperate climate, needs of heat for flushing are higher in populations from higher altitude and latitude. However there is little knowledge about flushing of Mediterranean species. The knowledge of geographic structure of the Iberian chestnut populations is interesting for the establishment of provenance regions, sampling and selection of conservation populations. The geographic variation of wild chestnut Iberian populations in bud break was studied in a provenance test including 19 provenance with two or three populations representing each provenance. It was planted in two sites in North-western Spain, with very different mean annual temperature and winter cold. Bud break was evaluated three consecutive years. Results showed a defined pattern of geographic variability: Northern populations flushed latter than the Southern ones. The mechanism responsible of this pattern could be directional selection favouring late break in areas with late spring frosts while in areas with summer drought, selection could favour early development before drought. The influence of cold and heat accumulation on bud break are studied as well temperatures causing frost damages.
GEOGRAPHIC VARIATION OF CASTANEA SATIVA WILD POPULATIONS FROM EXTREME EUROPEAN CONDITIONS EVALUATED IN A NURSERY EXPERIMENT

Forest species have a geographic structure revealing adaptation to climatic conditions. Geographic variation in adaptive traits as bud set, flushing and growth are well known for Northern species but little is known about the Mediterranean ones. The long range distribution of Castanea sativa indicate the probable existence of some adaptive variation among populations from extreme conditions. However, as the species has been artificially introduced in some European areas there is a question about the existence of adaptations to local environments. The variation in flushing, bud set and height growth between six European populations originating from areas of extreme climatic conditions of Spain, Greece and Italy was studied during one year in a nursery provenance–progeny test. Each population was represented by 26 open pollinated progenies in an experiment with 20 random complete blocks, one tree plot. Populations from Greece initiated growth earlier, followed by populations from South Italy and South Spain, while populations from North Spain and North Italy initiate growth later. Height growth of the Northern populations was higher than growth of Southern ones. Temperature and drought at the origin seem to be the factors responsible of variability among populations in growth initiation and growth height. Cessation of height growth occurred early in the summer before initiation of day length reduction. The North Italy population, representing a central European climate, ceased growing later than the other five populations originating from Mediterranean conditions. Differentiation among populations in bud set and height was more important than differentiation in flushing. These patterns of geographic variability are opposite to patterns of geographic variation of conifers from North temperate and boreal regions. Within population variation was significant for all traits but was more notable for flushing and height growth than for bud set.
Very little data is available for nucleotide diversity of genes involved in quantitative traits in natural populations of plant species. These genes, in particular those affecting adaptive changes, should be an essential component of future breeding and conservation programs. We quantified the levels of nucleotide diversity, and analyzed the structure and extent of linkage disequilibrium in 17 candidate genes of wood quality traits, that can also be linked to adaptive traits in maritime pine. The material included 13 provenances that were sampled over the whole natural range of the species. PCR fragments (300 to 600 bp) were sequenced on haploid tissues (megametophytes), allowing to detect a total of 210 polymorphic sites (SNPs and INDELs). There was on average 1 polymorphic site every 80 bp in coding regions and 1 every 25 bp in non-coding regions, but the amount, type and distribution of these sites were highly variable between genes. Two genes (a laccase and 4CL) showed significantly higher nucleotide diversity. A rapid decrease of LD was observed between polymorphic sites within most genes, while all sites of one laccase gene were in complete linkage, consistently with a much lower haplotype diversity than for the other genes. Differentiation at the nucleotide level between provenances (Nst) ranged from 15% to 37% among genes, indicating a much higher spatial structure than commonly observed for neutral markers in this species. Moreover, significant deviations from selective neutrality or demographic equilibrium hypotheses were observed for 3 genes among 6 for which sample sizes and diversity were high enough to apply neutrality tests. Tajima's D and Fu's Fs were of different sign and magnitude, indicating that both directional and balancing selection might have shaped the evolution of diversity within those genes. The significance of these results for future association studies in maritime pine is outlined.
VERTEBRATE-DISPERSED FRUITS IN REFUGIUM AND RECOLONIZER POPULATIONS: WHO FITS BETTER FRUGIVORE DEMANDS?

The large-scale migrations of plant populations during Quaternary climate changes have been assumed to be a catalyst for microevolutionary differentiations, particularly those related to dispersal efficiency. However, few empirical studies have asked if northern "recolonizer" populations produce better adapted propagules than populations in southern glacial refugia. Particularly, it has never been addressed if animal-dispersed fruits of northern populations may have been shaped by frugivore selection. We compare fleshy fruits from numerous populations across the European distribution range of the bird-dispersed tree Alder Buckthorn (Frangula alnus, Rhamnaceae). Populations vary significantly in most of the studied morphological and chemical fruit traits. We report patterns of variation and relate them to fruit selection cues of animal dispersers and the hypothetical postglacial colonization history of the populations under study.
The main objectives are to analyse how the environment during sexual reproduction affects the climatic adaptation of Norway spruce, with respect to: Selection processes, acting on gametophytes during male and female meiosis (meiotic drive), pollen tube growth, megaspore degeneration, fertilisation and embryo competition. Environmentally induced gene expression (genomic imprinting) during the reproductive process specifies the production of particular gene products (proteins, enzymes or regulatory molecules) shaping the expression of adaptive traits in the progenies.
The investigation of cytogenetic mechanisms of woody plants adaptation to stressful conditions is the vital question in forest plant genetics. At present the cytogenetic mechanisms of adaptation in coniferous trees have been studied in details. It has been shown, that their general adaptive mechanisms to stress are the increase of number of nucleolar organizer regions (NOR) in chromosomes, the increase of genome size due to amplification of some genes, the appearance of ring chromosomes and additional B-chromosomes and the presence of persistent nucleoli in stages of metaphase – telophase of mitosis. There is much less publications touching upon a subject of the adaptations in deciduous woody plants to stressful conditions. The following factors of adaptation are discussed there: tissue mixoploidy (in elm, poplar, aspen), the presence of B-chromosomes (in cases of considerable anthropogenic pollution). During several years (1994 - 2001) the study of cytogenetic characteristics of seed progeny of three deciduous woody plants – Querqus robur, Betula pendula, Ulmus laevis - was carried out in districts of Voronezh city and in Voronezh region, which differed in degree of anthropogenic pollution. We found the following mechanisms of adaptation in above species to stressful conditions: 1. The intensification of reparative activity may be observed in meristematic cells. The cytological manifestation of this phenomenon is the increase of chromosomal bridges in the spectrum of pathological mitosis comparing with the disturbances including lagging chromosomes in anaphase and in metakinesis. The checkpoint reparation of genetic material may be also activated, that is expressed in prolongation of mitosis stages. 2. The time of pathological mitosis peaks, which are subjected to diurnal rhythms and provide the normal tissue morphogenesis, is shifted with the retaining of number of such peaks during the day. 3. The changes of nucleolar characteristics include: - the increase of NOR number in chromosomes (the large number of cells with two or more nucleoli in nucleus appears in tissues); - the activation of transcription of previously worked NOR, which is expressed in the increase of nucleoli size, in the appearance of high-active types of nucleoli and in the growth of nucleoli heteromorphism); - the presence of persistent nucleoli in metaphase, anaphase, telophase of mitosis, which is provided by the puffing of ribosomal genes in these stages. 4. The change in diurnal rhythms of mitotic activity and variation of maximums number of dividing cells during the day may be observed in meristematic cells. 5. The growth of mitotic activity (simultaneously with the increase of cell number with the disturbances in mitosis) and the change in time of passing of the mitosis stages by the cells also provide the adaptation of deciduous trees in cytogenetic level. The data of this kind may be used for selection of stable genotypes and maternal trees, producing seeds with such genotypes, for making the stable plantations in extreme conditions of cities, and will allow to make a basis of cytogenetic monitoring of the environment pollution using the woody plants.
STUDIES OF THE SIBERIAN LARIX SPECIES IN RELATION OF THEIR TOLERANCE TO OXIDATIVE STRESS: MOLECULAR BIOLOGY AND CELL CULTURE APPROACHES

Abstract. The study was focused on genetic polymorphism of Siberian larch species (L. sibirica, L. gmelinii, L. x czekanowskii) using (A) polymerase chain reaction and direct sequencing of trnK intron of chloroplast DNA (cpDNA) and (B) long-term callus cultures. Established in the present work heterogeneity of trnK intron nucleotide sequence for 5 Larix species (L. sibirica, L. gmelinii, L. x czekanowskii, L. decidua, L. sukaczewii) studied so far makes this cpDNA fragment suitable for species differentiation. Comparative analysis of cpDNA polymorphism in L. sukaczewii allows it to be referred to separate species. A special work was carried out to reveal intraspecific polymorphism of trnK intron sequence in L. gmelinii. The characterization of this cpDNA fragment in this species is of special interest because of relatively high tolerance of L. gmelinii trees in comparison with other Larix species to oxidative stress induced by such an industrial pollutant as fluorine. Using in vitro long-term callus cell cultures there was studied also the possibility of differentiation of species and populations of Larix sp in terms of oxidative stress tolerance. Such a cell culture system may be of considerable interest both from the point of view of investigating intra- and interspecies polymorphism in larch in respect of sensitivity to fluorine as a widespread industrial polluting agent and from the point of view of simulating oxidative stress tolerance mechanisms in Larix species representatives, in particular, investigation of possible genetic consequences of the impact of industrial fluorine-containing emissions on larch cell genome. A number of questions and problems in the application of molecular biology and cell biology methods to study hybridization of siberian larch species, evolutionary significance of this genetic process and possibilities to improve larch trees based on natural populations are discussed. The financial support of INTAS (Grant 2001-0358) is acknowledged. Key words: chloroplast genome, trnK intron, Larix, callus culture, oxidative stress tolerance
GENETIC VARIATION WITHIN PINUS HALEPENSIS MILL. PROVENANCES GROWING IN DIFFERENT MICROENVIRONMENTS IN ISRAEL

We tested whether the F1-offspring raised from seeds collected in natural native Israeli and in overseas populations of Pinus halepensis Mill. planted under semi-arid environmental conditions exhibit genetic diversity and structure similar to those of their parental origin populations. Allele frequencies and genotypes of trees that had survived since planting 15 years ago, and those in the natural populations of origin were determined by two different methods: protein polymorphism by means of starch gel electrophoresis of enzymes, and genomic DNA polymorphism by means of the RAPD and PCR methods. The result of isoenzyme analysis showed that Percent polymorphic loci (P%) ranged between 12 and 44% in the natural populations and between 40 and 48% in the populations growing under the stressful environment. Mean value P% increases from 31% in natural forests to 44% in the Yatir populations and, consequently, the mean observed heterozygosity increase from 0.096 to 0.152. RAPD analysis showed that gene diversity (h) rose from between 0.264 and 0.316 in the natural populations to between 0.406 and 0.431 in Yatir. The calculated linear regression between allozyme heterozygosity and gene diversity resulted in $r = 0.690$, PR<0.001, n=5. Dry-land reforestation in places outside the natural distribution area of a species can be looked upon as peripheral populations with their higher genetic diversity conferred by selection, and thus having more resistance to extreme conditions. Therefore, such reforestation should be treated as a biogenetic resource available for rehabilitation and restoration of damaged Aleppo pine ecosystems.
ASH VARIABILITY IN NATURAL PLANTATIONS AND PROVENANCE TESTS OF UKRAINE

During the last ten years we learned interspecies variability of common ash (Fraxinus excelsior L.) in natural plantations and provenance tests. Investigation of provenance tests of European ash created in Trostyanets forest enterprise in 1930 were carried out to study interspecies variability in conditions of left-bank forest-steppe of Ukraine. The seeds obtained from 51 forestry enterprises represent all of the Ukrainian geographic zones: 11 populations from mixed forests (Polissya), 32 ones from forest-steppe and 8 ones from Steppe zone. The total area of the test is 2,18 ha. Studies of intraspecific variability of common ash were carried out with the use of standard biometrics and cytology methods in provenance tests. Silvicultural and taxation analysis allows distinguishing the following five climatic types of this species in Ukraine: polessky, western forest steppe, right-bank forest steppe, left-bank forest-steppe and ravine steppe. The best parameters of growth and productivity under conditions of D2 are typical for right-bank forest-steppe European ash, its average stock consists 472 m3/ha. The best quality of trunks has the populations of ash right-bank forest steppe. The high quality of trunks and preservation of trees ash are observed for the populations from left-bank forest-steppe and ravine steppe. The poor-quality (stem forking, water-shoot, the causes of cancer, frost-cleft) and curvature of trunks within the framework of ecological and geographical plantations of European ash differs the polessky and western forest steppe climatic types. The populations from polessky climatic òóðå differentiate most poor-productivity and poor-parameters of growth also. No impact of the parents stands productivity (their site index) on the progeny drowth was found. The Cytologic peculiarities of vegetative bud apexes were studied. A preliminary analysis of anatomy and morphological peculiarities of apex meristems allows, in total, to found three basic apex growth cone form types: 1. flat form, described in literature, 2. protuberant form 3. cupola-shaped form We have described the two last forms. The types occur with different frequency within the geographic zones. But a flat form is more frequent in populations of Steppe origin (85%), protuberant one is the most typical for populations from Forest-Steppe (88%), and cupola-shaped one – for populations of Steppe origin (76%). Diameters of apical meristem (D) increase clinally from Northwest to Southeast. No such trend occurs for diameters of subdistal zone. The latter character correlates with stem forking frequencies. Irrespective of geographic origin, the trees with stem forking have the larger diameter sizes (d) in comparison with trees without forks. The maximal frequencies of forking and the largest diameters of subdistal zone were found in provenances from Mixed-Forest zone. So the tendency to develop the stem forking is genetically inherited, but not the result of the damages of apex meristems by low temperatures, which is frequently mentioned in silvicultural literature. The type of bark is the good diagnostic criterion for the efficient estimation of the genetic variety populations. 6 different bark patterns were found on the trees of the provenance test: 1 type. Fine-slated; 2. type Columnar- slated; 3 type. Tabular –laminated; 4 type. Unruffled; 5 type. Fine- crack; 6 type. Large-scale-crack. Types 1, 2 and 4 are rare in the Western Forest-steppe and in Mixed-Forest zone. Some correlations between bark types and stem quality parameters have been found.
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COMPARATIVE DYNAMICS OF DIFFERENTIATION AT QTLS AND ADAPTIVE TRAITS UNDER BALANCING AND DIVERSIFYING SELECTION

Genetic differentiation in a set of populations under stabilizing and diversifying selection was investigated at two levels: QTLs coding for a trait, and the trait itself. A quantitative model with additive effects was used to link genotypes to phenotypes. No physical linkage was introduced. Using an analytical approach, and in the case of multiple diallelic QTLs, it was shown that the differentiation of the adaptive trait (QST) was dependent on the differentiation at the QTLs (GST), but also on the within (θ;w) and between population (θ;b) disequilibrium. We demonstrated that the discrepancy between the disequilibria (θ;b - θ;w) was the key factor involved in the difference between QST and GST. We showed by simulations that the difference (θ;b - θ;w) is still responsible for the discrepancy between QST and GST in the case of multiallelic QTLs. Simulations were used to study the effect of selection intensity, variance of optima among populations and migration rate for an allogamous and predominantly selfing species. Contrasting dynamics of the genetic variability at markers, QTLs and trait were observed as a function of the level of gene flow and diversifying selection. The highest discrepancy between QTLs and the trait occurred under highly diversifying selection and high gene flow, a situation that is likely to be frequent for many adaptive traits in forest trees. Furthermore, diversifying selection might cause substantial heterogeneity for differentiation of QTLs, only a few of them showing allelic differentiation, while the other behaving as neutral markers.
DIVERSITY IN UKRAINIAN NATURAL OAK POPULATIONS BY BARK STRUCTURE

Investigations in bark structure variability in Quercus robur natural populations in north-eastern part of Ukraine (Sumy, Kharkiv and Poltava provinces) and Quercus petraea natural populations in Crimea were carried out during inventory of in situ gene conservation units in 2001. The regions observed belong to Forest-Steppe, Steppe and Crimea Mountain natural zones. The work was carried out in the scope of the project «Genetic Resources of Broadleaved Species in Southeastern Europe» funded by the Ministry of Finances of Luxembourg and coordinated by IPGRI. 27 sample plots were established in 17 Q. robur gene reserves and plus stands and 2 sample plots were established in 2 Q. petraea gene reserves.

Most of the oak stands represent separated forest massifs, which we provisionally can consider as populations. In some cases several observed stands are located in the same forest massif. Diameter at 1.3 m, trunk quality category, health state, growth class by Kraft and bark type were determined for all trees in the upper storey on the each plot. A modified Iyevlyev (1972) classification was used for bark structure investigations. We proposed a scale giving the possibility to show any bark type in numerical expression. 6 bark types (3 main and 3 intermediate ones) from the thinnest (1) to the thickest (6) were discriminated: 1 – scaled bark. The thinnest bark (about 1 sm), separated by not deep longitudinal and transversal cracks from 5 to 15 mm larger on tablets. The scales is very easy dissevered; 2 - scaled-striated bark. Thequite thin bark, like lime bark. In longitudinal direction the bark superfisied separeted by net of narrow cracks. The transversal cracks are not so strongly marked as of 1 type.Intermediate type between 1 and 4.; 3 - scaled-pectinated bark. The bark of medium thickness, separated by system of not very deep and quite broad longitudinal and transversal cracks to areas, like trapezium in section. The surface additional separated by not deep longitudinal and transversal crasks on tablets.Intermediate type between 1 and 6; 4 – striated bark. The bark of medium thickness (about 2 sm), separated by marked longitudinal long and not broad cracks. The transvercal cracks are slight; 5 - striated-pectinated bark. The bark of medium thickness separated by system of longitudinal and transversal (not so strongly marked) quite thick cracks to areas, like trapezium in section. Intermediate type between 4 and 6; 6 - pectinated bark. The bark of medium thickness (about 3 cm), coarseness-cracked, separated by system of deep and quite broad longitudinal and transversal crasks to areas, like trapezium in section. Bark is very hard. The layers different dissevered. The high level of variability on the individual tree level within populations by bark structure has been found. The all 6 bark types were represented in 11 Q. robur sample plots from 29. Five bark types were represented on 10 plots and four ones - on 6 plots in Q. robur stands. Only 3 bark types represented in both Q. petreae stands. Three groups of Q. robur populations were discriminated by the ratio of different bark types and geographic location: North group, Southeastern group and Southwestern group. The North group, where trees with striated and striated-pectinated bark types predominate, comprises 3 populations (4 sample plots) from Sumy regions and 1 from Kharkiv region (located close to Sumy province). The Southeastern group, where predominate the trees with scaled-pectinated and striated-pectinated bark types, comprises 2 populations (7 sample plots) from Kharkiv region. The southwestern group, where predominate the trees with scaled-pectinated and pectinated bark types, comprises 6 population from Poltava region (12 sample plots). Four stands separated by the bark type ratio from any group. As to Q. petreae, trees with scaled and scaled-striated bark predominate in both stands. Some positive correlation between bark structure and diameter of tree was found in majority of stands (r = 0.31 - 0.66). The trees with thickest bark predominate in more productive stands. Reference: Ievlev V. V. The forms of English oak by bark //Tr. Voronejskogo Gosudarstvennogo zapovednika. - Vyip. 18,1972 – pp. 54-64
VARIABILITY OF STOMATAL CONDUCTANCE AMONG FULL SIB PROGENIES IN BEECH FAGUS SYLVATICA L..

The response of stomata to key environmental parameters has been generally studied on individual leaves or seedlings under controlled conditions, by means of infra-red gas analysers or porometers. This restricts the analysis to 10-20 plants in most cases. For population variability studies, large numbers of individuals have to be analysed. An assessment of the variability in this functional trait at population level would be of great relevance in light of the foreseen increase of drought periods under global change scenarios. We analysed the response to drought of full sib seedlings of European beech (Fagus sylvatica L) from seven families from a controlled cross. We measured the weight loss of each individual potted plant under drought in measured experimental condition (temperature and humidity). We estimated stomatal function parameters fitting a mechanistic model to the weight loss data. Significant differences among families were found. The genetic control of stomatal functions was estimated using the observed differences among families.
VARIABILITY OF STOMATAL CONDUCTANCE AMONG HALF SIB PROGENIES IN SILVER FIR (ABIES ALBA).

The response of stomata to key environmental parameters has been generally studied in individual leaves or seedlings under controlled conditions, by means of infra-red gas analysers or porometers. This restricts the analysis to 10-20 plants in most cases. For population variability studies, large numbers of individuals have to be analysed. An assessment of the variability in this functional trait at population level would be of great relevance in light of the foreseen increase of drought periods under global change scenarios. We analysed the response to drought of half sib seedlings (open pollinated) of silver fir from seven mother plants. We measured the weight loss of each individual potted plant under drought in measured experimental condition (temperature and humidity). We estimated stomatal function parameters fitting a mechanistic model to the weight loss data. Significant differences among families were found. The relationship between leaf area and conductance was studied. The genetic control of stomatal functions was estimated using the observed differences among families.
ECOTYPICAL DIFFERENTIATION AND INCIPIENT SPECIATION AT BOTH QUANTITATIVE TRAITS AND NEUTRAL MARKERS IN CEDRELA ODORATA

There are still very few studies which have examined the patterns of genetic differentiation in quantitative traits and molecular markers among populations of the same species. We investigated the genetic population structure, genetic architecture, and the degree of population differentiation in marker loci and genes coding quantitative traits among 29 populations of the endangered species Cedrela odorata in Central America and Mexico. Detailed analyses of quantitative trait and molecular markers divergence revealed a strong differentiation between the populations of the Atlantic coast of Panama and Costa Rica (high rainfall non-seasonal wet forest) as compared to the rest of Mesoamerica. The study suggests that a new subspecies is present in the Panama isthmus. An apparent explanation for the latter could be the pressure by selection in the very humid areas, adaptation to environments with a high humidity throughout the year and to the high competition with vegetation of very humid and pluvial forest that has considerably greater height and diameter than dry forests. Keywords. - Geographic variation, FST, QST, Cedrela odorata, natural selection.
SPECIFIC SERPENTINE TOLERANCES HAVE REPEATEDLY EVOLVED IN CERASTIUM ALPINUM DURING THE COLONIZATION OF SCANDINAVIA

Serpentine soils are rich in nickel and magnesium. In Scandinavia, Cerastium alpinum is one of the most common plants on these soils. Earlier enzymatic studies of serpentine and non-serpentine populations of C. alpinum have shown that this plant colonized Scandinavia both from the east and the west at the end of the last glaciation. The two genetic lineages seem to come into contact in a hybrid zone in northern Scandinavia. Here we have compared the effects of nickel and magnesium on the growth of plants from one serpentine and one adjacent non-serpentine population in the west, in the east and in the proposed hybrid zone. Seedlings were placed in solutions with different concentrations of nickel and magnesium. We used high and low levels of these elements in a full factorial experiment and the seedlings were arranged according to a randomized block design. The serpentine populations showed higher tolerance to nickel and magnesium stress than non-serpentine populations. However, the degree of tolerance differed among the serpentine populations. The mode of tolerance for a serpentine population seems to be correlated to the specific characteristics of the soil of that population. These results suggest that C. alpinum is not constitutively adapted to serpentine. Serpentine tolerance in C. alpinum is rather likely to have evolved locally in each site during the postglacial colonization of Scandinavia.
BIOMETRICAL EVALUATION OF GENETIC DIVERSITY IN NURSERY-GROWN CORK OAK SEEDLINGS (QUERCUS SUBER L.) WITHIN PROVENANCE HETEROGENEITY USING EARLY LEAF CHARACTERS

Under the funding of European Union project FAIR 1 CT95 0202 and Microaction B7 4100, an international network was set up to sample cork oak germplasm from provenances in the seven Mediterranean countries representative of the natural range of the species. Most of this germplasm was grouped by families and was germinated and grown under homogeneous conditions in a commercial nursery in Portugal. The measurements, made at 10-12 months after sowing, involved stem (height and branching) and leaf characters (area, maximal width, maximal length and perimeter). The data analysis is still underway and the present communication concentrates on the definition of leaf variable. Thus the present biometrical approach supports the notion of a high genetic differentiation within provenances in this species.
ISOLATION AND MAPPING OF PUTATIVE ADAPTATION SPECIFIC OSMOTIC INDUCED EXPRESSED SEQUENCE TAGS OF THE OAK GENOME

Pedunculate (Quercus robur L.) and sessile oak (Q. petraea (Matt.) Liebl.), the two main European oak species are difficult to differentiate at DNA level despite the apparently different morphological features. A neutral marker which helps to distinguish between the two species has recently been found (Muir, 2000). The two species prefer distinct microclimatic condition Q. robur moist while Q. petraea dry environment. It was assumed that in this adaptation process, genes responsible for osmotic regulation of the cell are also involved. Thus, genetic markers based on such genes may help to elucidate the differential adaptation of the two species to the environment. Therefore, several putative osmotic regulated cDNAs were isolated by subtraction hybridisation using Q. petraea suspension culture cells. Full-length information of the cDNAs was obtained by means of PCR based cDNA library screening. Based on the sequence information of the cDNA clones, genomic DNA of 8 parental oak plants corresponding to 5 families (3P x A4; 11P x QS29; 27P x QS28; QS21 x QS28; QS34 x QS29) was investigated at the 3' end of the selected genes to identify single nucleotide polymorphisms (SNPs). Each of the 24 genes studied provided several SNP positions useful for mapping purposes. Two crosses, one intraspecific (3P x A4) and one interspecific (11P x QS29), were analysed for the inheritance of the identified SNP markers in the full mapping family. SNP detection was done on an ABI 3100 Genetic Analyser using a 22cm array and POP4 polymer system. To the best of our knowledge this is the first attempt to position active genes on the linkage map of Quercus.
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VARIABILITY IN SUSCEPTIBILITY TO INK DISEASE IN EUROPEAN CHESTNUT.

Sweet chestnut (Castanea sativa) is widely distributed from England to Turkey in orchards and in mixed or pure forest stands. Intraspecific genetic variation and population differentiation has been demonstrated with isoenzyme markers. However, little is known about genetic variation in adaptive traits, and in particular, disease resistance. One of the major and widely distributed chestnut diseases in Europe is ink disease caused by Phytophthora cambivora and P. cinnamomi. These soil-borne pathogens infect the root system and colonise cortical and cambial tissues up the stem for several centimeters, resulting in tree wilting and death. Recently, high sweet chestnut mortality caused by ink disease has been reported from different areas in Europe and is likely to be associated with favourable environmental conditions. Among the possible control measures, selection and breeding for resistance are primary goals to reduce risk of disease in new plantations and nursery stocks. In orchards, interspecific hybrids (Castanea sativa x C. crenata or C. mollissima or the reciprocal) are known to be tolerant and are used as rootstocks. However, breeding for resistance in Castanea sativa requires knowledge of the extent of variability in susceptibility of cultivated and wild populations. Within the European research programme CASCADE, we have studied the variability in Phytophthora susceptibility among and within chestnut populations from different geographical regions (Sicilia and Piemont in Italy, Galicia in Spain, Gloucestershire and Suffolk in England and the Cévennes and the Maures in France) and with different domestication levels (naturalized populations, coppice and orchard, in this case, only the grafted varieties were studied). Experiments were carried out using the same methodology in two different laboratories: 6 populations (26 trees per population) from Italy and 2 from Spain were studied by the Italian team, 6 French and 2 English populations were studied by the French team. Excised shoots from each tree were inoculated by inserting a mycelium plug of P. cambivora onto a wound. After one week’s incubation in controlled conditions, the lesion length was measured, and used to evaluate chestnut susceptibility. In France, the experiment was repeated a second year for a smaller number of trees. Tree susceptibility estimated in the first year was well related with susceptibility estimated a year later. Analysis of variance showed that a highly significant variability of tree susceptibility occurred between and within regions. There was a significant effect of population on lesion length but no consistent ranking of the domestic levels was observed among the different regions. However, in three regions among five, orchard populations exhibited a lower susceptibility to P. cambivora than naturalised or coppice populations, suggesting that the selection of grafted varieties for their fruit quality was, in these regions, directly or indirectly associated to a selection for ink disease tolerance. The variance components of susceptibility were estimated using sums of squares of the «tree» and «population» effects. The ratio of var pop/(var pop + var tree) was 88% and 85% in Galicia and Sicilia respectively, 100% in Piemont, and 74% and 68% in the Cévennes and in the Maures, respectively. These high levels of tree variance components showed the wide variation in Phytophthora susceptibility among trees from the same region. Results from the genetic studies of the same populations will provide us with an estimation of their genetic diversity and of the potential for breeding chestnut in order to improve ink disease resistance.
PHENOTYPIC VARIATION OF LEAF MORPHOLOGY AND BUDBURST IN SOME ELM CLONES

In the frame of the EU project RES GEN CT 96-78 some of the partners have constituted clonal collections for germplasm conservation. It was considered of interest to assess their adaptability and, therefore, phenotypic plasticity. A particular attention was turned to the timing of vegetative budburst, because it was considered of primary concern for evaluating the probability of risk of spring frost damages and for verifying the existence of a relationship among budburst and attractiveness toward elm scolyts, vectors of DED, or with the susceptibility to DED. Leaf morphology is usually strictly connected with the plant growing environment, so to a change of growing site or to a changing climate it could correspond a leaf morphology variation. The aim of this paper was the study of the timing of bud burst and leaf morphology of some elm clones originated in Italy and France, cultivated in Italy, in the same site, in the same conditions.

MATERIAL AND METHODS
For the present study a sample of 42 field elm clones (Ulmus minor Mill.) coming from South, Central, North Italy and France planted near Firenze (Italy) at the same site, according to complete randomized block design, was considered. Leaf morphology
In June 2001 for each clone four short shoots deriving from four different ramets were harvested. On each leaf was measured the typical parameters suggested by Richens (1983) and Jeffers (1999) for comparative studies among elms. Then, two ratios were considered: a leaf thinness index, obtained from the ratio between the length and the breadth of the leaf, and a tooth depth index calculated by the ratio between the depth of the tooth and half of the breadth of the leaf. One-way ANOVA was calculated in order to establish the effect of the clone and of origin of the clone.

Phenological notations
Budburst phenology was weekly observed on all the ramets during the spring 2000 and 2001, according to 5 step scale (from 1= dormant buds to 5= two leaves or more are completely spread out). Overall variance was calculated for each day of observation. Three criteria were considered for analysing data: the day of highest variance for each year (criterion 1), the number of days from 1 January until each clone has reached the phase 3 (= bud scales are open and extremities of the first leaves are visible at the apex of the buds) (criterion 2) and thermal time (accumulated day degrees >5°C received by the plants from 1 January to the date of budburst) (criterion 3). One-way analysis of variance (ANOVA) was calculated in order to establish the effect of the clone and of origin.

RESULTS
Leaf morphology
Significative differences were found among teeth breadth (North Italian are shorter than Southern), length of the petiole (French are shorter than Northern and Central Italian) the tooth depth index (Southern are deeper than the others), thinness index (leaves of Southern clones are thinner than the others). Deeper teeth and thinner leaves could be an evolutionary adaptation to a warmer climate.

These morphological parameters are proved to be functional for species identification (Richens, 1983; Jeffers, 1999), but they demonstrated scarcely useful for describing variation within U. minor clones grew in the same site. This could be explained by a scarce variation within these traits, or, conversely, an extreme plasticity of leaf morphology.
Phenological notations
Post hoc comparison, calculated by mean of Duncan test, of the four provenances of the clones always leaded to the same result in both the years: flushing is significantly earlier in elms from Southern Italy and later in those from France, while plants from North and Central do not differ among them but are significantly different from those of South Italy and France.

In 2001, characterised by a particularly mild winter, the thermal time resulted 60-75% higher respect 2000. Despite this increment, the date of flushing remained unchanged, or showed, at least, a little bit earlier. This results suggest that in the trial site the chilling requirement is not completely satisfied for any of the provenances of the clones (Murray et al., 1989).

The early flushing of southern clones, is not univocally reported by bibliography related to different tree species (Wuehlisch et al., 1995; Falusi and Calamassi, 1996). The growth of trees of different latitudinal and/or altitudinal origin in a certain site colder or warmer than the original, could bring to different effects on flushing depending upon where on the curve thermal time-chill days the plant will lie in the new site (Murray et al., 1989).

The present deficiency of knowledge on dormance characteristics and on its evolution in gen. Ulmus does not allow a deeper interpretation of these results. Future researches should focus on these issues in order to assess the ability of this species to adapt to a changing climate.

References
F1-offsprings rose from seeds collected in natural native Israeli and overseas populations of Pinus halepensis Mill. where planted in 1985 in two provenance trials. One of the trials was planted under drought prone environmental conditions (The Yatir Forest), imitating ecological changes resulting from global climate change. We tested whether these 13-year-old offspring populations exhibit genetic diversity and structure similar to those of their parental origin populations. We also measured several eco-physiological properties to elucidate eventual differences between provenances in their response to drought. After 13 years, in 1997, average survival rate of the 23 provenances was 73.09% ± 13.50, ranging between 41.67% and 91.67%. Survival rate in 2000 declined to 34.83% ± 19.76, ranging from 0.0% to 59% because of two successive most severe winter drought ever measured of only 144 and 155 mm rainfall in winter 1998/99 and 1999/2000, respectively. Genetic diversity was determined in 1997/1998 by means of isoezymes analysis using starch gel electrophoresis, and genome DNA analysis by means of the RAPD based PCR method. The percentage of polymorphic loci increased from an average of 40% in the natural populations to 46% in the F1-offspring populations planted in Yatir. The average number of alleles per locus (A/L) rose from 1.3 in the parental populations to 1.5 in the offspring populations and, consequently, the observed heterozygosity rose from 0.049 and 0.115, respectively in the parental populations to 0.160 and 0.144, respectively in the Yatir populations. Average observed heterozygosity rose from 0.118 to 0.167. The largest changes in diversity, heterozygosity and fixation index occurred in the population that had originated in the highest and wettest environment, whereas minor changes occurred in populations that had originated in relatively drier environments. Analysis by means of RAPD based PCR showed that gene diversity (h) rose from 0.264 and 0.303, respectively, in the parental natural populations to 0.431 and 0.406 in Yatir. The calculated linear regression between allozyme heterozygosity and gene diversity resulted in R = 0.690, PR<0.001, n=5. Eco-physiological measurements revealed significant differences among and within sites between provenances in properties such as: Average predawn needle water potentials (-Mpa); Average daily transpiration rates (l day-1 per tree); Photosynthesis (µmoll/m-2/s-1) and Water use efficiency. Planting in dry land areas, outside the natural area of distribution of a species, can be looked upon as man mad peripheral populationes sensu Safriel et al. (1994). They will have higher genetic diversity conferred by selection pressure that eliminates the homozygous and favours the heterozygous genomes, thus, having more resistance to extreme conditions. Therefore, such plantations should be established and treated as a biogenetic resource available for rehabilitation and restoration of damaged natural or planted Aleppo pine ecosystems.
BLACK ALDER GENETIC RESERVES IN UKRAINE

Black alder occupy about 247 thousand ha, about 4.1% of the forest area of Ukraine. The species is natural propagated in the Ukrainian dampish and nasty soil with flowing subsoil waters. The optimum conditions for Black alder set in Polissya region. Species is valued as timber trees - wood suitable for plywood manufacture, as well as the stands of this species have also the great recreational and environmental importance. Black alder is one of by-roads of breeding in Ukraine. It should be noted that the intraspecific variation of Black alder in Ukraine is still not clearly understood. The best way to maintain the genetic diversity in forest tree species is conservation in-situ. Forest genetic reserves are the main way of conservation of forest genetic resources in population. In the early 1980’s on the base of pilot survey there was selected 15 Black alder timber-land genetic reserves (the total area is 192.1 ha) which is located in the Ukrainian Carpathians, Polissya and in forest-steppe region. In Polissya (Volyn region) was selected 8 plus trees. The objects had been selected during the 1983’s. Over a period of 18 years we conducted an inventory of the genetic reserves aiming at the assessment of their condition and functional potential. This material was compared with the preceding investigation dates regarding of the stands growing in Lebedins’ky forestry of Sumy region (located between 34o27’ N and 50 o38’E) and in Lubens’ky forestry Poltava region (located between 32o59’ N and 50 000’E). The work was carried out in the scope of the project «Genetic Resources of Broadleaved Species in Southeastern Europe» funded by the Ministry of Finances of Luxembourg and coordinated by IPGRI. The genetic reserves are mature 68-year age stands now. Oldest stands have the vegetative origin. The 35-year age stand of this species was surveyed in 2001 year in Lubens’ky forestry. A suitable stand has been identified, as a gene reserve and officially registered. During the inventory the permanent plots had been installed. The following marks were estimated the quantitative and qualitative features, bark type diversity at the population level. Lebedin site condition can be described as the marsh clay (D5) and Lubny - as wet clay (D4). The ground vegetation is relatively pure and contain much less then 10 species in 35-year stands grasses, the oldest stands is more rich by the numerous shrubs. Black alder stands are usually pure. Fraxinus excelsior L., Acer platanoides L., Ulmus glabra Huds., Betula pendula Roth. grows together with Black alder in the second storey as well as an understory in Lebedins’ky forestry. In comparison to first study, the content of ash, maples had been increased along the range with oak-ash stand. The old Lubens’ky stand is mainly pure, the single trees of Acer negundo L. and Ulmus glabra Huds is vegetated. Salix alba L. is the rare tree in composition of the cultures, Ulmus glabra Huds is met sometimes in second storey. Black alder is quickly growing valued species. The examined stands are high productivity. According to the inventory carried out in 2001, the mean growing stock of the Black alder of Lubny stand is 556.7 m3/ha, of Lebedin stand is 465.7 m3/ha. There values are respectively 1.5 times higher then the averages reported for these stands in 1983 year. In addition the Black alder forests of Poltava region is more productive against Sumy region on 14 % (in concordance with forest inventory data of the Ukrainian Forest Fund). On the average in the genetic reserves for this time the mean height has been increased of late years approximate at 5m, the mean diameter – at 10 cm. The interspecific variation in populations for the mean diameter is in average 19-20%. Productivity of the 33-year old is 350.3 m3/ha. Natural regeneration is preferable and it is the key method for given specie in Ukraine. Trees successfully propagate by the sprout, root-offspring. The most rapid and successful regeneration method is planting, one was using for forest plantation creation. In the stands selective thinning has been carried out. On the basis of the results of breeding inventories the qualitative marks of the stands was been designated. At the present time the Lebedin genetic reserve are presented by the multi-stem trees, within the oldest Lubny reserve such trees are much fewer. More then 30 per cent of trees had been concerned to the highest
selected category, just as this category of trees had been discovered only 13.7 per cent. More then the half of stands are forms by the trees of a first and second Kraft class. The advantage by the vital condition the 33 years Black alder stand was three times better. The most common defects are the curvatures and water shoots. Simultaneously the bark types were investigated. There were exposed following types: Å- smooth; LP- large-scale plates (deeply fissured into large, flat and flaky plates ~1.5-3 cm); SP- small plates (superficial fissured into minor flaky plates ~0.5-1 cm); LF- large-scale fissured (middle thickness bark, separated on areas ~ 1.5-3 cm by system of vertical and horizontal fissures of middle depth); SF - small fissured (separated by shallow fissures on small flaky plates ~ 0.5-1 cm); S - smooth bark (separated by faintly conspicuous not deep) As the result in alder stand the LP, SP, LF types are predominated, in the oldest stand 93% trees have LP bark. The work on inventory other Black alder genetic resources has not begun yet. A database (national catalogue) of genetic resources is developing.
THE STUDIES OF INTRASPECIFIC DIVERSITY OF SCOTS PINE IN THE PROVENANCE TESTS IN UKRAINE

Most of the information about among-population variation derives from investigations of provenance tests, which have played a great role in forest research. Provenance experiments contain genetic entries whose seeds were collected in geographically different localities and should represent a much larger area than an individual stand. The objects of our studies were Scots Pine provenance tests of the 1st and 2nd generations in Sumy and Kharkiv regions of Ukraine. 1st generation of provenance tests had been created in 1928-30 years. The Scots Pine provenances from 224 forestry enterprises located in 92 regions of former USSR are represented here. The 2nd generation of provenance tests was created in 1965. Seeds for creation of the 2nd generation had been harvested in the provenance tests of 1928-30 years. Here are represented 20 provenances of Scots Pine. We studied the populations that maximally representing the natural distribution range of Scots Pine in the former USSR: Arkhangelsk, Polotsk, Bobruysk, Bryansk, the local (Trostyanets), Vyatka, Saratov, Busuluk, Kokchetav, Akmolinsk, Georgia. The forest valuation and cytological signs were investigated. The cytological investigations of Scots Pine’s pollen and seeds in the 1st and 2nd generations of provenance tests were carried out in first time in Ukraine. We have obtained the following results. Heredity of geographic variability of Scots Pine biometrics traits was confirmed. The local and Bobruysk populations had best growth and state in 1st generation of provenance tests at the age of 70; the worst ones had Kokchetav and Arkhangelsk. The local and Bobruysk provenances were best by the forest valuation characters in the 2nd generation at the age of 30; the worst ones were Kokchetav, Arkhangelsk and Georgia. The considerable intraspecific variability of Scots Pine pollen by germination, fertility, morphometrical and histochemistry indexes were found. Best viability of pollen was obtained in the local, Polotsk, Bobruysk, Bryansk and Georgian populations. The coefficients of variation of morphometrical indexes were low (from 1.91 to 11.69%), that confirms more stability of these descriptions for different provenances of Scots Pine. The values of pollen grains general length are diminished from North to South. The maternal effect in inheritance of geographic variability of Scots Pine seed’s germination was observed. The coefficient of correlation between indexes of absolute seed’s germination in 1st and 2nd generations was 0.856. The intraspecific variability of mitotic activity of apical meristem of germinated seeds of Scots Pine’s provenances was defined. The dynamics of mitotic activity for the different populations in 1st generation has the considerable discrepancies. The maximum mitotic activities of apical meristem of germinated seeds in 1st generation were higher, than in 2nd generation for all populations. The differences of mitotic index’s dynamics in 2nd generation were reduced, that can be caused by the cross-pollination. The average level of mitotic activity of cells is decreased from South to North. The studies of intraspecific variability allowed selecting in the provenance tests the best populations by the complex of descriptions. Those are provenances from western part of European Russia and from Byelorussia: Polotsk, Bobruysk, Bryansk (all these population are from the central part of the natural distribution range of Scots Pine in Europe). Accordingly, a well-grounded system of signs for definition of biological correlate between forest valuation and cytological levels of studies of the Scots Pine’s populations was worked out. The selected best climatypes could be recommended to creation of high productivity Scots Pine’s forest stands.
INVESTIGATIONS OF THE REGULATION MECHANISM FOR THE ADAPTATION TO DROUGHT STRESS IN NORWAY SPRUCE

Adaptation of Norway spruce to extreme climatic conditions is generally believed to develop in the course of many generations due to the interaction of several evolutionary processes (mostly selection). Recent results with Norway spruce have indicated that climatic adaptation can occur in only one generation in response to the climatic (temperature/photoperiod) conditions during sexual reproduction. In order to establish the mechanisms underlying this short-term adaptation, possible gene regulations are analysed by means of innovative molecular genetic methods such as differential display of mRNA (DDRT-PCR). This technique allows to identify genes which are differentially expressed under designated conditions among identical genotypes. It has been suggested that plants have common mechanisms in their physiological responses and their tolerance to drought. First results show that also in Norway spruce a transcription factor named DRE (dehydration-responsive element) plays an important role in drought-induced gene expression.
The European white elm (Ulmus laevis) is a component of riparian forests in central and eastern Europe, thriving in wet soils which are periodically flooded. Owing to its riparian habit and to the drainage and deforestation of floodplains for agriculture, U. laevis populations are often small and relatively isolated. In the future habitat destruction and, in the eastern part of the range, Dutch Elm Disease, appear likely to fragment the species further, leaving the remaining populations at risk from genetic drift and inbreeding. In 1999, a common garden experiment was established at Uppsala, Sweden, with genotypes taken from Sweden, France, Germany and western Russia, in order to gain an insight into the amount and distribution of adaptive variation in the species. Putative adaptive traits, including bud set and bud flushing, growth rate (height) and leaf senescence have been recorded over two growth seasons. We hope that our results will be useful in the design of both in-situ and ex-situ conservation strategies for U. laevis.
GENETIC DIFFERENTIATION OF TREE POPULATIONS IN THE SOUTHERN URALS (RUSSIA)

The level of differentiation between samples of the main tree species of the Southern Urals (Pinus sylvestris L., Picea obovata Ledeb., Larix sussczewii Dyl., Betula pendula Roth., Quercus robur L., Acer platanoides L., Populus nigra L.) was investigated by means of isoenzyme analysis. All parameters of the differentiation (Fst = 2.9 – 6.4 %) were in a range found between tree populations in Europe, as it has been reported in literature. Samples of naturally regenerated seedlings of an introduced tree species, Acer negundo L., have a significant higher degree of genetic differentiation. The most evident differences were obtained for parameters of genetic variability of separate loci (Ho = 0.095 – 0.643, Aat-1; Ho = 0.220 – 0.463, Aap-1; Ho = 0.111 – 0.333, Aat-2). The influence of stochastic and deterministic factors on population structure and colonization dynamics is discussed.

Sessile (Quercus petraea) and pedunculate oaks (Quercus robur) are two important forest tree species that naturally hybridise with each other. However, they show several morphological differences at adult stage. The goal of our present study is the development of diagnostic PCR-based markers to easily discriminate the two species at an early stage. Considering that both species show different responses to anoxic stress, our focus went on the identification of differentially expressed genes in the roots under anoxic conditions.

Sessile and pedunculate oak clones were grown under hydroponic conditions for two months under non restrictive O₂ concentrations as a control, followed by an anoxic stress using a mix of 97% nitrogen and 3% O₂. We measured the kinetic activity of different enzymes involved in the response of anoxia on root tissues at six different stages (0-48h) as a control to select the best stage (24 hour) for RNA extraction. We used cDNA-AFLP to detect differentially expressed fragments between the two species and under different periods of O₂ restrictions. Among the 4000 amplified fragments obtained by this technique, 5.5% and 4.1% were specific to the sessile and pedunculate oaks, respectively, and 3% showed differential expression between stressed and non stressed conditions.

Forty one fragments were sampled, cloned, sequenced and compared to Genbank. Overall, 65.8% of the sequences were similar genes of known function involved in several biological processes (metabolism, cell wall, signal transduction, genes and proteins expression, stress, transport), 22.0% corresponded to unknown Arabidopsis genes, and 12.2% did not present similarity in public databases. The resulting expressional candidate genes will be ultimately validated using reverse northern or RT-PCR.
The white willow (Salix alba L.) is a common species of European floodplain forests. This species is cultivated or reproduces naturally in a vegetative or sexual way along most European rivers. In its natural environment, white willows are found together with many other willow species and hybridizes quite easily. This research group, as a partner in the DYNAMO project, concentrates on polymorphism detected in the chloroplast and mitochondrial DNA. The plastid genomes have a uniparental inheritance, a clonal mode of evolution and have a slow evolutionary rate. These properties are particularly interesting for studies of plant evolutionary biology. This present work mainly focused on different haplotypes of white willows detected in European populations. Short fragments (150-1000bp) of cpDNA and mtDNA were amplified with various conserved primers, that were chosen from a database. The preliminary part of this study produced molecular markers specific to species susceptible to crossing naturally with the white willow in different regions of Europe. Individuals containing plastids of white willows were selected with these markers and cpDNA markers for different hyplotypes were developed. SNPs were detected by PCR-RFLP or multiplex PCR on agarose gels. Indels were detected on acrylamide gels. The genetic homogeneity or heterogeneity of populations in terms of haplotypes was characterized and rare haplotypes were detected. These results combined with those of the other groups of the DYNAMO project can flag important issues for the conservation and management of willow plantations and natural populations in Europe.
EVALUATION OF GENETIC DIVERSITY IN SARDINIAN POPULATIONS OF ALEPPO PINE: IMPLICATION FOR MANAGEMENT AND EXPLOITATION OF ECONOMICAL RESOURCES

The knowledge about the genetic structure of plants and animal populations allows correctly evaluating the strategies for the conservation of biodiversity, for repopulation and for breeding. Prerequisites for this kind of analysis are the availability of a set of neutral genetic markers and of a genetic map to describe the amount of genetic variability at the "general" level; however, to develop strategies for sustainable development it is of the greatest importance the evaluation of the "adaptive" genetic variability: this can be achieved by the availability of genes, or molecular markers linked to the genes, responsible for traits of importance for survival. Aleppo pine has been present in Sardinia as an endemic species since at least the 15th century, when the existence of the two natural populations remaining at our time was historically attested. Sardinian populations represent, from the genetic point of view, peripheral populations: the importance of semi-isolated populations resides in the fact that they offer the opportunity to look for specific allele combinations which predominate for specific blocks of genes, adaptive to ecological stresses. The assessment of the degree of variability of genes related to drought tolerance in Pinus halepensis would therefore assume importance for the in situ conservation of this ecologically dominant, across the whole Mediterranean area, conifer species. In this poster the amount of «neutral» genetic variability present in the two natural populations of Pinus halepensis has been assessed by means of SSR and AFLP markers and compared to that of other Mediterranean populations. We have found quite high values for Fst (0.21 for microsatellites, 0.12 for AFLPs) suggesting that a good amount of interpopulation variability is still harboured by this species. Phylogenetic analysis based on Nei’s genetic distances clearly differentiates the Western (Spain, Morocco) populations from the Sardinian and Greek ones. A Mantel test (genetic distances vs. geographic distances) was not significant, thus suggesting that isolation by distance is not the only responsible for the differences observed. Genes which are thought to be responsible of drought tolerance in the Angiosperms (rab, myb, DREB) are currently being isolated to be used to estimate the «adaptive» variability.
STRUCTURATION OF THE GENETIC DIVERSITY OF AN AGROFORESTRY SPECIES, THE SHEANUT TREE (VITELLARIA PARADOXA), USING RAPD AND CHLOROPLAST MICROSATellite MARKERS

The sheanut tree (Vitellaria paradoxa) represents one of the major species in agroforestry parklands in the sub-Saharan zone. It has a socio-economic (oleaginous product) and ecological importance and is concerned by domestication and conservation issues. However little is known on the pattern of the genetic diversity within the species. In order to better address these issue, a study of genetic diversity was realised with a sample of 148 individuals representing 14 populations of the natural range, with two molecular markers, RAPD and chloroplast microsatellites. RAPD, using 15 primers, gave 66 markers. The analyses showed an high within population genetic diversity. A very weak structuration was observed and was explained by a strong gene flow. With chloroplast microsatellites, on the nine primers used, four presented polymorphic loci and their association gave ten chlorotypes. Eight populations, forming the west group, exhibited 7 chlorotypes while the centre-east group, representing by 6 populations, possessed only 5 ones. A stronger structuration was observed with this marker. The analyses allowed to discuss the history of these populations in connections with refuge theory and permitted to give recommendations for the conservation and valorisation of these species.
In response to phytosanitary, commercial, legal or ecocertification concerns, numerous actors of the forestry industry are looking for a reliable technique permitting to control wood origin. The important database on chloroplast DNA variability of European white oaks has allowed to precisely map this variation, which is characterised by a strong geographical structure throughout the European continent. In our study, molecular markers adapted to the analysis of oak wood were developed. The potential of dry wood for genetic analyses was first defined. The DNA isolated from wood is largely degraded and present in low amounts. Genotyping success rates increase on fresh sapwood and if targeted sequences are short and present in high copy number per cell. Molecular methods were then developed to characterise wood haplotypes. Five different PCR-RFLP combinations can be used for that purpose. They allow to check statistically the conformity of the haplotypes detected on wood samples with those encountered in the hypothetical region of origin. The last studies concerned two different applications of these molecular methods for wood genotyping. The first is an industrial application where the method was adapted to the control the geographical origin of oak wood used to make barrels. The second is in the field of paleogenetics and archaeology where the typing of ancient oak wood allowed to better understand the action of Man on oak populations and the past use of this resource.
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ISOZYME VARIATION IN QUERCUS SUBER L. RELATED TO HISTORICAL FACTORS AND INTROGRESSION WITH QUERCUS ILEX L.

Data from an isoenzimatic study carried out in 31 natural populations of cork oak (Quercus suber) have been employed to investigate the relationship between the values of diversity parameters and the postglacial history of the species, and to detect possible imprints of recolonization. Populations located in recently colonized regions showed a loss of genetic diversity, mainly a lower allelic richness. Genetic drift associated to founding events and bottlenecks seem to have determined the structure of scattered and small stands. A parallel study of chloroplast DNA variation detected the occurrence of genetic exchanges with Quercus ilex in 7 out of those 31 populations. The possible influence of this citoplasmic introgression on the nuclear variability has been assessed. Using the isozyme data, an assignment test for the individuals of the introgressed populations has been performed, taking as possible origins "pure" (with suber haplotypes) cork oak populations and 5 Q. ilex stands from which isoenzimatic data are available. The test clearly assigned the trees with ilex chloroplast to Q. suber populations. Even an increased diversity was not observed in these stands, as could be thought in case of incorporation of foreign genes. Citoplasmic exchanges do not correspond with exchanges at nuclear level. Introgression with Q. ilex follows a geographic pattern, being found in peripherical regions where usually cork oak constitutes fragmented stands far from the main range. This points to the idea of more facilities for hybridization and higher probabilities of allele fixation when the size of the population is low, as could have been happened during the glaciation. The historical factors mentioned above would have determined the occurrence of citoplasmic exchanges as well as the patterns of nuclear variation of Q. suber.
DEVELOPMENT OF SPECIES-SPECIFIC MOLECULAR MARKERS FOR WILLOW SPECIES HYBRIDIZING WITH WHITE WILLOW (SALIX ALBA L.) IN EUROPE.

The white willow (Salix alba L.) is distributed all over the floodplain forests of Europe. It hybridizes with a number of other species of the genus Salix, present in the same alluvial environment. The morphological characterisation of these hybrids is relatively difficult. Salix rubens is the most common hybrid described in central Europe. Individuals of S. alba, S. fragilis, S. pentandra, S. vitellina and S. babylonica show the same chromosome number and were investigated in particular. However, S. triandra, S. vimminalis and S. purpurea have also been studied because of their ability to hybridize with either S. alba or S. fragilis. This partner in the DYNAMO project concentrates on polymorphism present in the chloroplastic (cpDNA) and mitochondrial DNA (mtDNA) of white willow and its hybrids. Because of the uniparental mode of inheritance of the plastids maternally inherited in this genus), their slow evolutionary rates and their clonal mode of evolution, they offer an interesting perspective for studies in phylogeny, plant phylogeography or interspecific gene flow. The aim of this study was to analyze phylogenetic relationships between these different species as well as to develop PCR primers allowing a reliable and rapid identification of hybrids. Combined "conserved" primer pairs were chosen from a database to amplify short plastid DNA regions. In this way, indels and SNP could easily be detected. Ten different regions of the large and short single copy region of the cpDNA and three of the mtDNA were amplified and sequenced. Species specific primers, for the species mentioned before, could be designed and used for the identification of hybrids as well as for selection of individuals in intraspecific studies.
TESTING THE MONOCLONAL STAND HYPOTHESIS IN POPULUS TREMULA L. NATURAL POPULATIONS

Populus tremula L. has been traditionally thought to establish new stands with very few viable seeds (often only one) by propagating vegetatively through root suckers. Isozyme patterns were studied in five Populus tremula stands in northern Spain to test the variability of trembling aspen in natural populations. A maximum of thirty trees per population were selected following criteria of maximum distance between individuals and canopy accessibility in an attempt to capture the widest possible range of variability. Ten isozyme systems were analyzed: LAP, GOT, ADH, SKDH, IDH, 6PGD, MDH, PGI, PGM, and ACPH. Plant material consisted of dormant buds. Thirteen loci exhibiting both high heterozygosity and allele richness were identified. Populations showed differences among them, as well as a low gene flow. Genotype analysis yielded several genetically different individuals in every populations, rejecting the monoclonal stand hypothesis in stands of natural origin.
JOINT ANALYSIS OF POST-GLACIAL MIGRATION IN TWO SOUTHERN BEECHES (NOTHOFLAGUS SPP.)

The genus Nothofagus is the principal component of southern South America Temperate Forest, which lies on both sides of the Andes Cordillera (Chile and Argentina). The modern distribution area of these forests was highly affected during Quaternary ages by glacial movement and climatic changes. As opposite to what happened in the Northern Hemisphere, glaciation in the South left many ice-free areas, where glacial refuges could have been located. Assuming a common life history, joint analysis of two inter-fertile important species, Nothofagus nervosa and Nothofagus obliqua, was carried out. Chloroplast DNA genetic markers were detected in 25 and 10 populations respectively using two primers (trnD-trnT and trnF-trnV) and two restriction enzymes through a PCR-RFLP technique. Five haplotypes were observed in both species from the three primer-enzyme combinations analysed. The geographic distribution of these haplotypes was highly structured independently of the species. In the sympatric areas on the eastern slope of the Andes the same two haplotypes were observed in both species defining two clearly separated geographic groups. Haplotype IV was present in only one population of N. nervosa from Chile located between the two groups. Two Pacific coastal populations of N. nervosa had each a different haplotype (haplotypes III and V) which in turn were not found among the other populations. These results suggest the existence of multiple common ice age refugia where both species survived the glacial period. Moreover, the extant populations in Argentina and those from the Andes in Chile are not the result of a post-glacial migration from coastal refuges as proposed in previous palynological studies. In the southern group only one population located west of the Andes showed the same haplotype than those to the east. Given the larger distribution of haplotype I in Argentina, we suggest that some of the glacial refugia must have been situated east of the Andes. Since most frequent haplotypes are shared by both species a long term hybridisation and introgression process could be assumed.
GENETIC DIVERSITY OF SCOTS PINE (PINUS SYLVESTRIS L.) POLISH PROVENANCES BASED ON RAPD ANALYSIS.

The high level of genetic diversity is one of the most important parameter in an adaptation strategy of forest ecosystems under changing environmental conditions. Coniferous tree species cover 78% of the forest area in Poland and they constitute the main wood resource for industry. The aim of this proposal is to analyse the interpopulation diversity among Pinus sylvestris provenances, using molecular markers as a tool. Then, the polymorphism (RAPD) was assessed to find out the most detailed pattern of nested relationships between the studied populations. This allowed us to start the phylogenetic study of pine in eastern European countries. Our results helped us to apply a molecular marker analysis for the selection of provenances characterised by a high genetic variability for future forest tree improvement programmes at the European level.
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CAN ALIENS BE CONSIDERED AS A THREAT FOR DIVERSITY IN ALLUVIAL FOREST ECOSYSTEMS?

Invasion of organisms is a frequent and natural process in riparian zones because of constant flows of water, nutrients and sediments, which periodically return portions of the riparian forests to early successional stages. But factors that support high diversity, in conjunction with river managements and forest fragmentation increase also susceptibility to invasion by exotic species, particularly when rivers are intensively managed, and forest is reduced to small fragments. The result is that floodplain forests are now subjected to both invasion by allochtonous, competitive species and considerable loss of natural diversity. The Japanese knotweed population of the Rhine valley is the subject of an investigation into its spread and further evolution along the interconnecting riverine forests of the floodplain. Fallopia taxa invaded the rivers entering the plain from the Vosges, some decades ago, and a particular high density is now found along the Thur, the Fecht, the Giessen and the Doller. Its invasion along these tributaries of the Rhine is observed as small patches along river banks and alluvial forests. Preliminary results indicate that different taxa predominate on different river systems. Only F. japonica var. japonica was found on the River Thur whilst on the Giessen near Selestat extensive growth of hexaploid F. x bohemica was found. However on the River Fecht, both hexaploid and octoploid F. x bohemica were found, as well as some spontaneously established second or third year Fallopia seedlings. Cytologically these plants were 2n = 88; 2n = 66; 2n = 75, a most interesting result, and were presumably the progeny of various crosses within and between the two ploidy levels of F. x bohemica there. This region of the Fecht may therefore be considered as a hot spot for hybridisation and the production of future new fertile taxa. Such fertile seeds might be implicated in a further, more aggressive invasion of alluvial forests and nature reserves along the Rhine. We may however consider the emergence of new (possibly endemic) species from these exotic invaders, as an enrichment of the alluvial flora, going some way to compensate for the loss of natural diversity caused by human activities in floodplains.
MITOCHONDRIAL DNA VARIATION AND THE EVOLUTIONARY HISTORY OF LARIX IN NORTHERN EURASIA.

28 populations of Larix were sampled throughout the Europe, Siberia and Far East. Six probe-enzyme combinations and two PCR-RFLP markers were used to study mtDNA variation. The number of haplotypes varies strongly among species, ranging from one (or two but with a predominant one) in "marginal" species (L. decidua, L. kaempferi and western L. sibirca) to up to thirteen in eastern L. sibirica. Most of the haplotypes were specific to distinct species. Haplotypes specific to western L. sibirica and haplotypes specific to eastern one were found in the north of Western Siberia, perhaps due to post-glacial dispersion of Larix from the refugia in the Urals and from some populations in the Eastern Siberia. On the same time it is unlikely that mountain area in Southern Siberia (Altai and Tuva) was a refugium for the post-glacial recolonization of the northern territory because haplotypes that are most common here are absent in the northern territory. In contrast with great homogeneity of populations of L. gmelinii and L. olgensis revealed by chloroplast microsatellite and AFLP, mitochondrial data show significant differentiation among them. Haplotypes that are most common in L. gmelinii were absent in L. olgensis and vice versa. Haplotypes that are specific for the putative introgression zone between L. olgensis and L. gmelinii are rather rare or absent in pure L. gmelinii and L. olgensis. These results suggest that unlikely to young L. sibirica – L. gmelinii introgression zone, L. olgensis and L. gmelinii came into contact much earlier or their divergence has not been completed yet.
DISTRIBUTION OF HAPLOTYPES OF WHITE OAKS IN CENTRAL BALKANS

CpDNA of white oaks from coastal Croatia, Bosnia and Herzegovina, Montenegro, Kosovo region, FYR of Macedonia and Albania were analysed with the same methods that were used in a previous survey of European oaks. Haplotypes characteristic for Balkan refugium were found, including haplotypes from Apeninic peninsula. There geographical distribution helped fill an important gap in the knowledge of postglacial migration of oaks in Europe.
The genus Quercus comprises between 300 and 700 species and is distributed throughout the northern hemisphere across the different continents. We investigated the molecular diversity within and among botanical sections of Quercus. CpDNA polymorphism was measured by sequencing the trnD-trnT cpDNA fragment in 54 oak species. The fragment comprised microsatellite regions, and diversity was analysed at the microsatellite regions and at the point mutations. (1) For the microsatellite regions: There were 5 microsatellites loci with poly-A (1-3 and 5th locus) and poly-T (4th locus). There were more repetitions (nucleotides) in species of North America than in others, with the red oaks exhibiting the highest diversity. The species distributed in North America can be distinguished from others in one microsatellite (4th locus). Nei’s genetic diversity within group varied from 0.2030 for Chinese white oaks to 0.4364 for white oaks in North America. Genetic divergence was the largest between the oaks in North America and the oaks from other continents and the smallest between the European white oaks and the Chinese white oaks. (2) For the nucleotide sites regarded as punctual mutations: Results obtained for nucleotide diversity were similar to those obtained for microsatellites. In addition the diversity within the Brachylepides subsection (species from the Yunnan province) was much larger than for the other groups.

These results together with the phylogenetic analysis confirm recent results about the origin and dispersion of oaks during the tertiary. Asia was most likely the origin for oaks of the Cerris section (including the Brachylepides subsection), from where they migrate westwards to Europe. The red oaks distributing uniquely in North America and the higher diversity in the North American white oak group suggested that North America was one of the origins of Quercus. Hence the two major botanical sections (Cerris and Lepidobalanus+Erythrobalanus) differentiated following a vicariance of an ancestral group forming the intercontinental mesophytic forest at the beginning of the Tertiary.

Keywords: trnD-trnT sequence, microsatellite locus, nucleotide diversity, botanical section (group) of Quercus.
In the context of global climatic change, temperate forest tree species will be faced to drier soil and air conditions. Growth, development, productivity but also wood quality of these long-lived organisms may be greatly affected. Adaptation of these species to future climatic conditions is important to consider for sustainable forest management. With this aim in mind, we are developing a research program focused on two adaptative traits: drought tolerance and date of bud bursting, respectively for maritime pine (the first conifer species used for reforestation in south west Europe) and sessile oak (one of the two most important white oak in Europe). A transcriptomic approach is developed to identify and characterize genes that are involved in each trait. For maritime pine, a global approach based on a massive EST sequencing from controlled and stressed root cDNA libraries, is used to construct a pine DNA chips (UNIGENE extracted from 20 000 ESTs). For sessile oak, we follow a targeted approach based on SSH (Suppressive Subtractive Hybridization) cDNA libraries representing different developmental stages during bud burst. In both cases, gene expression is studied at the transcriptome level. RNA probes to be hybridised on high density filters will be prepared (i) from needle, stem and root of controlled and stressed seedlings for two maritime pine provenances presenting a difference in their physiological response to drought and (ii) from six developmental stages in oak. Sequence diversity will be estimated on natural populations for the most regulated genes. This poster will present preliminary results and discuss the potential use of these genes for assessing adaptative diversity.
DETECTION OF QUANTITATIVE TRAIT LOCUS (QTLs) FOR THE RESPONSE OF WATER USE EFFICIENCY TO HIGH CO2 IN A PEDUNCULATE OAK FULL-SIB FAMILY

The response of water use efficiency (defined as the ratio biomass accumulation over water use) to rising atmospheric CO2 concentration ([CO2]) will affect the adaptation of trees to future environmental conditions. At leaf level, intrinsic water use efficiency can be estimated either by using gas exchange measurements (ratio of net assimilation rate (A) over stomatal conductance (gw)) or based on the carbon isotope composition of leaf material which provides time-integrated estimates of A/gw. The objective of this experiment was to assess the effects of elevated [CO2] on WUE and to detect possible QTLs related to d13C and physiological parameters and to their responses to high [CO2]. Two-year old pedunculate oak (Quercus robur L.) trees from a full-sib family (200 genotypes) were grown in greenhouses and exposed to either 360 or 700 mmol mol⁻¹ [CO2] for one growing season. Several gas exchange measurements and samplings were performed during the summer. d13C was measured on leaves and wood cellulose produced during the second and third growth flushes. Water use efficiency was higher for trees grown at elevated CO2, mainly due to a decreased stomatal conductance. QTLs were detected for gw and d13C on linkage group 5. Additional traits that will also be used for QTL detection include stomatal density, and activities of enzymes related to the primary carbon metabolism (photosynthesis and photorespiration).
Within the studies on forest biodiversity evolution and management, a particularly effective approach is represented by the evaluation of the responsiveness of tree populations to climatic variations. At this regard, the setting up of dendroecological monitoring networks allows the identification of the environmental gradients that may have affected the distribution of single species and/or the composition of entire coenoses. We developed a first attempt for a dendroecological network in Central Italy, which has already provided many interesting information on some aspects of the adaptive capacity of Italian forests. We determined the critic periods for tree growth and evaluated the relative importance of the climatic variations in relation with the geographical characteristics of sites (mainly the altitude) and time windows (from decades to centuries). We focused our interest on water stress, the main limiting factor for plant growth in the Mediterranean region. We actually aim to genetically characterize populations that underwent water stress situations differing in duration, intensity and phase. At the same time, our study enabled us to identify the oldest forests in Italy, with ages more than 3 centuries. We noted that responsiveness of these populations to changing environmental conditions varied in time, with special regard to the older trees which endured the Little Ice Age and several different selection pressures during their long lives. An evaluation of the genetic diversity among the different generations of a forest thus appears of a fundamental importance in order to ascertain the real adaptive potential of a coenosis as a whole, providing necessary information for a sustainable management of forests and the natural resources of a region.
SOMATIC STABILITY OF MICROSATELLITE LOCI IN EASTERN WHITE PINE, PINUS STROBUS L.

Variation at microsatellite loci was studied among and within clonally-propagated individuals of Eastern white pine. Total DNA was extracted and assayed from gamete-bearing tissue (megagametophytes) located on six different branch positions on each of twelve individual genets. No within-individual variation was observed among twelve loci studied. Estimates of numbers of mitotic cell divisions required to produce the tissue used as the source of genomic DNA were obtained by combining tree growth and anatomical data. This allowed calculation of upper bound estimates of numbers of mutations per locus per somatic cell division. Somatic mutation rate estimates fell into the range of 10^-7 to 10^-6, and are thus substantially lower than those published for genomic microsatellite mutation rates in other plant species.
SPATIAL GENETIC STRUCTURES AND ESTIMATION OF POLLEN FLOW IN A MIXED FOREST OF SESSILE OAK (QUERCUS PETRAEA) AND PEDUNCULATE OAK (Q. ROBUR) IN SWITZERLAND: AN ANALYSIS OF NUCLEAR MICROSATELLITES

Sessile oak (Quercus petraea Liebl.) and pedunculate oak (Q. robur L.) are the most frequent oak species in Switzerland. They are inter-fertile, but the degree of natural hybridisation is still a matter of debate. In a species-rich mixed forest with about 20% oaks, all mature oak trees within a plot of 9 ha were mapped. The trees were assigned to one of three demes based on their leaf morphology: 79 % Q. robur, 19 % Q. petraea and 2 % putative hybrids. These trees were genotyped at four nuclear microsatellite loci to investigate the spatial arrangement of genetic variation. We applied techniques of point pattern analysis to estimate the spatial arrangement of the mother trees and to examine the spatial distribution of genetic information in the population. Nearest neighbour distances were employed to evaluate their spatial point pattern against one generated from spatial randomness. At least 30 acorns from each of 10 mother trees were genotyped using the same four loci. Exclusion probabilities were estimated and paternity analysis was performed to identify the pollen donor trees. In addition, the spatial distribution of the pollen donors and the direction of pollen flow within and between the two species were analysed. Parameters of gene flow via pollen were studied using spatial techniques supported in a geographical information system (GIS) developed for the genetic resources in the study area. All four microsatellite loci were highly polymorphic in the three demes. We observed a total of 7–25 alleles per locus. Observed heterozygosity (Ho) varied from 0.783 to 1.000 per locus and deme, and expected heterozygosity (He) ranged from 0.759 to 0.902 per locus and deme. No significant deviations from Hardy-Weinberg structures were observed within the demes. Accordingly, fixation indices were low (0.004 for Q. petraea and 0.026 for Q. robur), indicating random mating within demes. The genetic information in the mother population does not seem to be randomly distributed in space. Distinct spatial genetic structures were observed, which were associated with species identity. The paternity analysis provided information about potential pollen donors with a high discrimination power when all four microsatellite loci were used. The pollen trees were distributed over the entire study area, but the most likely fathers were found to be at a maximum distance of about 100m from the mother tree.
GENETIC RELATEDNESS AND PARENTAGE ANALYSIS IN A TWO-COHORT MIXED OAK STAND IN CENTRAL SPAIN

Lack of fructification can threaten the survival of forest systems based on natural regeneration. This is particularly true in populations located in the edges of the range of distribution of the species, where environmental conditions are not usually suitable for reproduction. This is the case of the study area, where two oak species (Q. petraea and Q. pyrenaica) coexist and a lack of fructification has been observed, in particular in Q. petraea, a species typical of central Europe temperate forests. In central Spain, the sustainability of oak forests is also endangered because of the fragmented and scattered distribution of these species. In order to determine the causes of scarce reproduction in oaks, we studied genetic variability and population structure in 96 Q. petraea and 83 Q. pyrenaica adult trees located in Montejo de la Sierra (Madrid), one of the most singular forest systems in central Spain, using five nuclear microsatellite loci. Also, the existence of a high inbreeding level in this forest, resulting in a lack of fructification, was checked. Inbreeding could also risk natural regeneration. With this aim in view, we sampled 44 Q. pyrenaica and 52 Q. petraea saplings located in a central subplot and a parentage analysis was made to determine the contribution of individual adult trees to the new generation and the patterns of gene flow within the forest.
GENETIC STRUCTURE AND PARENTAGE ANALYSIS IN SHRINKING OAK STANDS

Vitoria-Gasteiz is located above a natural aquifer. Discriminant analysis on inventory data from oak stands surrounding the town shows three predominant structures, very much dependent on the levels of underground water: mixed Q robur-F excelsior-A campestris- Populus sp stands in the lower (wetter) lands, Q faginea stands in the upper (drier) lands, and mixed Q robur-Q faginea stands in intermediate areas. It is well documented that the Q robur mixed stands have shranked during the last 50 years due to agricultural competition, and there are reasons to believe that most of these lands were once part of a pedunculate oak forest that dominated the landscape. Besides their small size, the genetic structures of these “island forests” have been very likely influenced by their long term use to provide fire-wood and grazing. Restoration efforts being carried out by local authorities might be hindered by at least three potential hazards: reduced genetic diversity within stands, disturbed genetic subdivision, and lack of new saplings (which could be related to difficulties in seed establishment and/or hybridization with the surrounding Q faginea). In order to help take correct management decisions, we have studied the genetic diversity and population structure at 6 microsatellite loci in 4 island forests and 2 gardens that comprise the majority of Q robur individuals in this area. Pairwise Fst analysis showed significant differences between the two garden areas (both with the lowest number of individuals); but it allowed to join two adjacent islands which formed one single forest not many years ago. The largest island forest was sampled twice, first within the Q robur mixed area and then within the Q faginea zone. Results show a strong genetic differentiation among the Q robur and the Q faginea stands; which is mainly due to the behaviour of just one SSR (AG36) with Fst values near 23%. Two other island forests showed 2 cohorts, the elder with Q robur trees above 100 years and the younger, more variable, with individuals ranging from 20 to 50 years. Parentage analysis of the younger cohorts using all Q robur trees as potential parents found no potential pair of parents and only found a few potential single parents. Analysis of contributions to total heterozigosity and allelic richness showed that the population at the greatest risk (due to the airport enlargement) is one of the most important from the conservation point of view.
FACTORS AFFECTING MARITIME PINE GROWING EFFICIENCY IN NATURE: SIZE AND VIGOUR, INTRASPECIFIC COMPETITION AND FAMILY STRUCTURE

Growing efficiency (GE) is defined as the ratio between individual-tree growth and the portion of ecosystem resources a tree uses. GE is an estimator of the ability of a given tree to growth using the limited resources available in the microenvironment where it inhabits. In this work, we studied the correlation between GE and several parameters related to size and vigour, intraspecific competition and family structure, the latter estimated using highly polymorphic nuSSR markers, in a native maritime pine (Pinus pinaster Ait.) stand located in Mediterranean Spain (Coca, Segovia). First, linear models were constructed to relate GE (estimated as the ratio between basal area five-year growth and horizontal crown projection) to size, vigour, and competition parameters, namely crown ratio (CR), diameter at breast height (DBH), total height (HT), crown competition at height (CCH), basal area at larger trees using DBH to define larger trees (BAL) and basal area except a given individual (BAT). Second, the residuals of the best model explaining the previous variables were computed, and a model including ten dummy explanatory variables (one for each sib-family identified within the stand using SSR markers), was constructed in order to analyse the main effect on GE of each sib-family. Only the crown ratio, a variable which represent the vigour of the tree, was significant to explain growing efficiency. Moreover, there was not a relationship between GE and the indexes used to describe intraspecific competition. Microenvironment variation (soil and water supply, mainly) not detected by competition indexes could explain these results. Contrasting with the lack of relationship among GE and competition, an important family effect, explaining around 50% of the residual from the previous model, was found. In fact, two families showed significantly higher and one significantly lower GE than the rest. The study of within-populations genetic structure using molecular markers and its correlation with quantitative traits in nature, based on the present results, seem promising tools to improve management and conservation of forest genetic resources.
CHARACTERISATION OF GENETIC VARIATION IN EUROPEAN COMMON ASH (FRAXINUS EXCELSIOR L.).

Knowledge of the genetic potential of Fraxinus excelsior L. (European common ash) populations in Ireland is essential for future breeding programmes and conservation management plans. Increasing interest in the species as a crop has will require data on its genetic potential. The overall aim is to provide a characterisation of ash through molecular genetic analysis of Irish populations. Analysis will be concentrated on natural populations as well as a provenance trial set up by Coillte at Drumsnea, Co. Cavan. Mircrosatellite analysis to look at variation within as well as between the natural populations will be presented. DNA sequences produced for the plastid DNA region trn L-F, atpB-rbcL and rpl 16. Variation between the sequences will be examined and primers designed for the amplification of microsatellite loci (SSRs). An overall investigation into the genetic variation in Ireland will be carried out using AFLP analysis, also a study on the geographic distribution of plastid DNA haplotypes will be carried out.
VARIATION AND POPULATION STRUCTURE FOR CONE, SEED AND PLANTLET TRAITS WITHIN AN ALEPPO PINE REGION OF PROVENANCE. IMPLICATIONS FOR SELECTION AND GENE CONSERVATION.

In Algeria, Pinus halepensis covers about 850 000 ha in coastal and inland areas where populations are subjected to various selection pressures. A few studies have shown a substantial geographic variability in phenotypes for adaptive and morphology suggesting geographic and ecotypic differentiation within the species. On the other hand, no genetic information was available for such traits at the within-population level. So, this work was intended to fill some gaps in this field whose importance for selection and conservation is evident. 49 open pollinated families of eight stands of Aleppo pine from the western coastal region of Algiers were included in the study. The prospected zone extends over about 15 km (as the crow flies) parallel to the Mediterranean coast and on about 3 km in depth in the back country. There was no significant differences between stands for most traits suggesting that no genetic differentiation between stands exists within the studied region. Isolation by distance was not as complete, and common pools of genotypes would be shared among stands at relatively similar frequencies. Conversely, within-stand (family) variation was high. In particular, heritabilities were strong for plantlet traits as the number of cotyledons ($h^2=0.57$), the length of hypocotyl ($h=0.56$) and the length of cotyledons ($h^2=0.84-0.88$). So, there was evidence for genetic structuring within stands, probably resulting from neighbourhood figures where genetic variability lacks. Parameters as size and location of stands may play a role in shaping genetic variation within stands. This variation was generally of lesser importance in small and/or coastal stands. Given the size of the studied area, it was not expected to find marked links between traits and geographic variables such as altitude or longitude; nevertheless, it was observed west-east clines of variation for 100-seed weight and wing width, parallels with an increase in annual rainfall from western to eastern parts of the zone. As a direct implication for selection, it may not be of interest to retain for seed use purposes stands or restricted areas when prospecting at a local scale; rather, priority should be given to selecting families and/or individuals. The substantial within-stand variability is a guarantee for buffering environmental changes. And, at the whole population level, because of the lack of differentiation among stands, it would be sufficient to preserve only a few of stands for ensuring efficient gene conservation. Key words. Aleppo pine, stand variation within a region of provenance, seed, cone, plantlet traits. Résumé. Pinus halepensis s'étend sur environ 850 000 ha en Algérie où se rencontrent des populations depuis les régions côtières jusqu'aux confins de l'Atlas saharien. Quelques études, à l'échelle de l'aire algérienne de l'espèce, ont montré une diversité conséquente de phénotypes pour des caractères adaptatifs et morphologiques, suggérant une différenciation géographique et écotypique au sein de l'espèce. Par contre, on ne dispose pas d'information sur la variabilité génétique au sein des populations. Aussi, ce travail se propose-t-il de combler quelques lacunes dans ce domaine dont l'importance pour la sélection et la conservation n'est pas à démontrer. 49 familles en pollinisation libre issues de 8 peuplements de la région côtière à l'ouest d'Alger constituent le matériel de base de cette étude. La région prospectée longe la côte sur une quinzaine de km et s'ensorce sur environ 3 km dans l'arrière-pays. Les résultats obtenus suggèrent une absence de différenciation entre peuplements à l'échelle de la zone étudiée. L'isolement par la distance peut être incomplet, et, par conséquent, un réservoir commun de génotypes est partagé par les différents peuplements à des fréquences analogues. A l'inverse, la variabilité génétique au sein des peuplements est conséquente, attestée par des valeurs d'héritabilité fortes pour les caractères de plantules. Cette structure de variation intra-peuplement traduit probablement
l'existence de structures de voisinage. Des paramètres comme la taille ou la localisation du peuplement peuvent jouer un rôle dans l'expression de la variabilité génétique y existant ; les peuplements de petite taille et les peuplements littoraux expriment un degré de variabilité moins important. Étant donné la taille de la région d'étude, il n'était pas attendu de liaisons marquées entre caractères et variables géographiques comme la longitude ou l'altitude. Cependant, des clines de variation ouest-est ont été observés pour le poids de 100 graines et la largeur de l'aile de la graine, parallèles à une augmentation de la pluviométrie annuelle. Comme implication directe pour la sélection, il peut ne pas être opportun de retenir un peuplement ou une zone de faibles surface à des fins de récolte de semences à l'échelle régionale. Il faudrait plutôt privilégier le choix de familles et/ou d'individus sélectionnés à l'échelle de toute la région. La variabilité génétique intra-peuplement est une assurance de pérennité contre les changements (naturels) des conditions environnementales en les tamponnant. Par ailleurs, à l'échelle de la région, en raison du manque de différenciation entre peuplements, la préservation d'un petit nombre de peuplements pourrait se révéler suffisante pour une conservation efficace des gènes présents dans la population. Mots clés : Pin d'Alep, région de provenance, variation entre peuplements, cône, graine, plantule.
CLONALITY IN NATURAL POPULATIONS OF THE WILD SERVICE TREE, SORBUS TORMINALIS

The insect-pollinated Sorbus torminalis shows clonal growth through root suckers. It is commonly believed that this species forms well delimited groups of trees (ramets) belonging to the same genet and that sexual recruitment in established populations is rare. While recent molecular studies on this species have revealed high genetic variation within populations, the influence of clonal growth on the spatial genetic structure has not previously been investigated. We mapped the position of all trees in ten natural populations (population sizes ranged between 13 and 143) from north-eastern Switzerland and determined their genotypes at six polymorphic allozyme loci. Again, populations had high genetic diversities (mean $\text{He} = 0.42$), which were slightly lower than the respective observed heterozygosities (mean $\text{Ho} = 0.43$; $P = 0.04$). This is indicative of an outbred mating system. Populations were characterised by (1) high numbers of genotypes that occurred only once and (2) distinct patches of recurring genotypes, i.e. ramets of the same genet (mean clonal diversity $M = 43\%$). Neither genetic diversity nor clonal diversity were significantly correlated with population size. Autocorrelation analysis of multilocus genotypes (Moran's I) revealed that clonal growth significantly influenced the spatial genetic structure of the populations at distances of about 15-30 m. In conclusion, clonal growth guarantees genet persistence and, hence, can aid in the maintenance of local genetic diversity even if sexual recruitment is low.
Le Sapin de Numidie (Abies numidica De Lannoy) est une espèce forestière endémique des monts Babors en Algérie. En Algérie, cette espèce a été introduite à titre expérimental dans quatre sites différents dans le but de constituer des échantillons de conservation ; dont la plantation de la forêt de Serraïdi. Cette dernière est l’objet de cette étude, qui a pour objectif d’évaluer les capacités de germination des graines et la production grainière des arbres, donc l’aptitude de ces derniers à la régénération naturelle. Les paramètre de vigueur (hauteur et diamètre) des individus échantillonnés ainsi que leur répartition spatiale ont été utilisés pour déterminer leur relation avec les caractères étudiés. Les résultats obtenus dénotent : - des aptitudes des arbres de cette plantation à la production de graines viables (le taux de bonnes graines de l’ordre de 55% et capacité de germination entre 4.44 et 62%) et à assurer le recrutement de nouveaux individus par régénération naturelle, contribuant fortement à la dynamique, au maintien et à la diversité de cette population et de l’espèce en général. - la capacité d’adaptation du Sapin de Numidie en dehors de son milieu naturel. Mots clés : Abies numidica De Lannoy, régénération, graines, plantation, endémique, adaptation, germination, dynamique.
BIODIVERSITY OF TYPES OF ECTOMYCORRHIZAE AND NATURAL REGENERATION OF SPRUCE

The limitations for natural regeneration of Norway spruce are classically compared to the temperature and light regime of the site. However, survival of shaded ectomycorrhizal trees has been proven to depend on the mycelial networks, connecting different sources of nutrients in a forest ecosystem, whereby different types of ectomycorrhizae have a different role in nutrient acquisition and translocation. In our complex study we have tested the possible interactions among mycorrhizae, light regime and survival of spruce seedlings on the plot. Types of ectomycorrhizae were studied in soil cores (274 ml, 0 - 18 cm deep) from 33 sampling plots from a young regeneration center from the permanent forest research plot on Pokljuka (Triglav National Park, 1200 m/s.l.), established in an autochtonous Norway spruce stand. Light regime, soil and natural spruce regeneration were studied on the same plots. In mycorrhizal samples all the roots were counted and types of ectomycorrhizae briefly characterised. Some taxa were verified by PCR-ITS-RFLP. Diversity indexes were calculated & all data were correlated (STATISTICA: basic correlations & factorial statistics). In total 51,049 root tips were counted, 1% of which were non-mycorrhizal, 63% were old unviable mycorrhizae & 36% were identifiable ectomycorrhizal root tips, forming 27 different types of ectomycorrhizae. Shannon diversity index for types of ectomycorrhizae was relatively high (2.06) with respect to the above-ground diversity of vegetation (1.7). Similar data were discussed previously. Selected genera of ectomycorrhizae were correlated positively with: i) E horizon (podzolization) (Lactarius sp., Q-type), ii) humus (Cenococcum geophilum) or iii) fermentation layer (O\textsubscript{I},O\textsubscript{II}, Elaphomyces sp.). The survival of natural spruce seedlings was negatively correlated with direct irradiation.
EFFECT OF STAND COVER, SOIL PREPARATION AND PROTECTION AGAINST GRAZING ON SURVIVAL OF CEDRUS ATLANTICA SEEDLINGS AT TALAGUILF (DJURDJURA, ALGERIA).

In situ seed sowing has been undertaken in different conditions of stand cover (clearing, border, under forest) soil preparation (lock-picking, superficial work and control) and protection against grazing (with or without). The results showed the best seedling survival at the forest border in combination with lock-picking and protection against grazing. Theses favorable microclimatic conditions to in-situ seedling establishment could provide an explanation to the patchy dynamic of recruitment observed in Cedrus atlantica stands, where few genotypes should be favored by their location at the border and/or a good seed production.
POPULATION GENETIC STRUCTURE AND MATING SYSTEM OF A TROPICAL TREE DICORYNIA GUIANENSIS.

Dicorynia guianensis is a canopy tree endemic to the tropical rain forest of French Guiana. We compared temporal and spatial genetic structures for maternal (chloroplast) and biparental inherited (microsatellite) markers in two different cohorts in order to infer processes shaping the distribution of genetic diversity. The study was conducted on a 40 ha study plot located at Paracou near Kourou (French Guyana) where 172 adults trees and 375 saplings were sampled. The species is low density (4 trees/ha) and distributed in imbricated aggregates of various sizes ranging from 100 to 400 meters. Both cohorts showed similar level of nuclear and chloroplastic genetic diversity. Genetic differentiation was more pronounced between spatial aggregates than between cohorts that could be produced by a restricted seed flow. Despite the spatial differentiation, the species was able to maintain high levels of diversity for maternal genomes, suggesting a rapid turnover of aggregates. The mating system could also affect the genetic diversity and then the evolution of the population. The species has single, bisexual flowers and thought to be pollinated by large bees (according to the floral syndrome). Open-pollinated progenies were collected on 20 adult trees that were the only ones producing seed in 2000. In total 203 seeds were collected and genotyped with the 6 microsatellite markers (Latouche-Hallé et al 2002). Two female parents produced enough seeds (33 and 37) to allow individual genetic analysis. We estimated single and multi locus outcrossing rate (ts and tm). We found an outcrossing rate of 0.91, that was a common trait to most of tropical trees. The difference (tm - ts) was not significant indicating that there are no matings among relatives, in spite of the aggregated distribution of D. guianensis. Using the neighborhood model (Burczyk et al 2002, we found that the proportion of pollen coming from outside the study stand amounted to 60%. Dicorynia, as observed in others low density tropical trees (Murawski and Hamrick 1991), tolerates significant selfing rate, but is also able to disperse pollen over long distances.
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POPULATION GENETIC STRUCTURE AND MATING SYSTEM OF KOOMPASSIA MALACCENSIS (LEGUMINOSAE) IN PENINSULAR MALAYSIA

Koompassia malaccensis is an important tropical timber tree species. Population genetics study for K. malaccensis was carried out using isozyme markers. Six populations, viz. Pasoh Forest Reserve, Sungai Lalang F.R., Sungai Menyala F.R., Bukit Bandi F.R., Jerangau F.R. and Bukit Lagong F.R. from Peninsular Malaysia were investigated, of which all are virgin jungle reserves. Leaf samples from about 40 adult trees were collected from each population for isozyme analysis. Three enzyme systems (Leucine aminopeptidase, Peroxidase and Uridine diphosphogluconate) yielded six loci were obtained of which five loci were polymorphic. Mean expected heterozygosity of K. malaccensis was rather high, 0.203. However, coefficient of differentiation between populations, $G_{ST}$ is very low, only 0.033. Likewise with the mean genetic distance, $D = 0.012$. No correlation was detected between estimates of genetic and geographic distances ($r = -0.0031; p = 0.5130$), indicating that these populations could have been continuous long time ago. Isozyme analysis on progeny arrays from ten mother trees was carried out to investigate the breeding system. Results showed that K. malaccensis has a mixed-mating system, with average multilocus outcrossing rate of 75.5%. Only one private allele was detected in Sungai Lalang population. Bukit Bandi was identified as a unique population through jackknife analysis. Implications of the study in conservation strategies are discussed.
ASSESSING THE AUTOCHTONOUS STATUS OF FOUR SILVER FIR (ABIES ALBA) STANDS FROM A BEECH DOMINATED FOREST FROM THE NORTHERN ITALIAN APENNINES.

Autochthonous populations have high conservation value for many reasons. They harbour information on the evolutionary history of the species and more in general are interesting as examples of populations that overcame the challenge of balancing the contrasting risks of inbreeding and outbreeding depression. Isolated population subject to drift and with a limited gene flow are also important as source of genetic resources that might have been lost in the continuous part of the geographic range of a species. Chloroplast microsatellite markers, known for their high level of variability, are suited for the detection of genetic relatedness of populations in general and in particular for assessing the autochthonous status of a population.

We used two chloroplast microsatellite loci Pt 30204 and Pt 71936 recently used in a European wide investigation of the species. Beside the four stands under investigation located in the Northern Apennines (Parma province, Italy) we used seven stands from the Italian Alps (3 from the Western, 3 from the Central and one from the Eastern Alps). Using filogeographic methods and the probability of assigning individuals to a population we estimated the autochthonous status of the four Apennine stands.
CLONE STRUCTURE IN POPULUS TREMULA

As a part of a large project investigating "Population biology of European aspen, Populus tremula", I have searched for suitable markers for clone identification in this species. P. tremula reproduces mainly through root suckering, and individual trunks (ramets) can be members of the same individual (genet). The clonal structure of aspen in different forest types (managed and old growth forest) is not well known, although it largely sets the stage for the occurrence of taxa specialised on aspen (insects, fungi and lichens). The sample trees for this study are situated in Kuhmo, north-eastern Finland, where extensive mapping of aspen has been conducted. The trees were assigned to different morphotypes according to morphological markers such as spring foliage colour, branching type and stem form. I have tested these trees with nine microsatellite loci originally developed for Populus tremuloides, a close relative (Dayanandan et al. 1998, Rahman et al. 2000). Preliminary results suggest that there are more clones than morphotypes both in old growth and in managed forests. Microsatellites were able to distinguish even morphologically similar clones. The size of the clones was quite small, app. only two or three trees per clone both in old growth and managed forests. Further analysis will show if the clonal structure in old growth and managed forests is different and what is the spatial distribution of the clones.
The present communication reports on the available data from a study started in 1993 with 24 contiguously distributed adult trees in a naturally regenerated stand located to the south of Lisbon. Male flowering in Spring and fruit production in Autumn were recorded on a 3-grade scale of intensities (from 1 as "poor" or "absent" to 3 for "heavy"). Phenology of male flowering was coded on an alphabetic scale related to the succession of developmental stages as described by Varela & Valdivieso (1996), and the survival of developing embryos in labelled branches was also recorded. Flower counts per catkin were also analysed. The average male flowering intensities among all trees were either "high" in 1993/6/8 (range 2.5-2.67) or "low" in 1994/5/7/9 (range 1.75-2.21). Some trees tended to be the highest producing irrespective of the year, others lowest, but a subset did not show any regular pattern. None did match all yearly plot modes through the whole observation period. The phenologies of male flowering were very different among trees, with a maximal difference of 30 days among anthesis termination dates. Catkin size and flower density were significantly different among trees. In general, the intensity of fruit production was on average lower than the male flowering intensity in the corresponding Spring season. This trend was more marked in some trees than in others. Highest fruiting intensities were observed in 1993/6 and lowest in 1994/7/9, with 1995 and 1998 lying in between due to uncoupling from the relevant male flowering intensities. In Spring-Summer 1994 the fruit development in labelled branches from 9 trees showed that on average there was a mortality of 80% before fruit maturation was accomplished. The present results show that cork oak has a very irregular reproductive behaviour, both with time and among trees, thus stressing the relevance of measurable indicators for the conservation and exploitation of gene resources in this species.
Combining different genotyping techniques to investigate taxonomic relationships in trees. An example in Cedrus species.

Cedrus forests are currently found in distinct geographical areas around the Mediterranean: Cedrus atlantica in North Africa, C. libani in Turkey, Syria and Lebanon and C. brevifolia in Cyprus. Both strong human impact and ecological requirements are responsible for a scattered distribution and some alarming levels of limited natural stand size. Species of the genus Cedrus are commonly used in reforestation programs in Europe (e.g. France). Paternally inherited chloroplast microsatellites (cpSSRs) and amplified fragment length polymorphism (AFLPs) were used to identify both the range wide and the within population patterns of variation in 16 populations: 11 from Turkey and Lebanon, 4 from north Africa and France and 1 from Cyprus. Size variants at 3 chloroplast DNA regions yielded 89 cpSSR haplotypes and 11 selective AFLP primer pairs generated a total of 101 polymorphic amplification products.. Both markers indicated a stronger within population diversity (Heterozygosity and allelic richness) in C. libani and C. brevifolia than in C. atlantica. Among population diversity estimates (Gst and Analysis of Molecular Variance) converged in both kinds of markers and indicated geographical structuring, contradictory to the accepted taxonomical nomenclature and to adaptive trait variability measured in common garden experiments. The Turkish C. libani populations were more strongly differentiated from Lebanese C. libani populations than they were from C. brevifolia. Seed transfer from Turkey to Cyprus and/or random genetic drift following deforestation in Cyprus and Lebanon could be responsible for this pattern.
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PRELIMINAR EVALUATION OF GENETIC VARIABILITY OF CRYPTOMERIA JAPONICA POPULATION IN AZORES ARCHIPELAGO

Cryptomeria japonica (L. fil) Don was introduced in S. Miguel Island, one of the island of Azores archipelago, in the middle of the 19th Century, as an ornamental tree, like other species introduced in Europe in those days. Later on, sugi was used all over the Archipelago as windbreaks and live fences, as well as to control soil erosion and protect natural water lines on steep hillsides. Its good adaptation and fast growth singled sugi out for forest purposes and rapidly superseded all the other species. Since 1934, sugi has been widely planted in monoculture. At present, sugi stands cover around 15 075 ha (22% of the total forest area), from which 10 600 ha are located in S. Miguel, representing 69% of the present total forest area of this island. Cryptomeria introduction in Azores Islands occurred in a period when genetic information was overlooked. There are no reports about the origin of seed used in Azores afforestation, and conscious that seed quality should not be left to hazard, the local government launched a Forest Breeding Program in 1998. Within this program, in order to evaluate the genetic basis of Azores sugi population and to compare it with natural populations, three provenance trials were established in the spring of 2002 in Faial, Terceira and São Miguel Islands. A previous characterisation of the height of the different geographical origins was done at the nursery, as expected the Azores origin plants had a better growth comparing with the Japanese, which showed a similar behaviour between the four breeding regions present in the trials. The provenance trials experimental design is also presented. The progeny of 30 plus trees was evaluated by the msp-PCR technique, to obtaining previous conclusions about the genetic variability of the breeding population.
THE SURVEY OF THE DISPERSION OF FOREST TREE SPECIES IN TIME

The survey is based on data from the Slovenian Forest Service. The average growing stock per unit (in each of ca 86,000 permanent forest units) for the forest tree species is recorded. The units have the average size of several hectares. Until now only the forest tree species were recorded which had the average growing stock larger than 1% of the average growing stock of all the forest tree species in the unit. In the next decade all the forest tree species will be recorded. At the Slovenian Forestry Institute we have incorporated the data into a geographical information system. The centroid of the unit represents a point in the map. The different colours represent different ranges of the average growing stock of a particular tree species in the unit. Until now we have only the first survey from 1999. With the next survey we will also show on the map the modification in the dispersion of forest tree species in time.
Quercus rubra L. is one of the most important timber and ornamental tree species from East North East America and was introduced in Europe in the 17th century. Only scarce information to date is available on the diversity of the introduced populations in comparison to that of the natural populations. We conducted a genetic survey by assessing chloroplast DNA polymorphism in both gene pools. The survey was based on a total of 259 oak trees from 55 Americans populations (AP) and 282 samples from 58 European populations (EP). Variation at five chloroplast DNA (cpDNA) fragments was studied using PCR-RFLP, resulting in the detection of 14 haplotypes in the two continents. Nine haplotypes are shared and two are private to the AP and three to the EP. In the AP one haplotype was present in 75% of the trees, and 11 present in 0.4-7.3% of the trees. Similar values were observed in the EP, with one haplotype present in 77% of the trees, and 10 in 0.3-9.6%. The most important result is the low genetic differentiation (Gst = 0.481±0.08) in the natural populations, in comparison to other Fagaceae. Data of pollen deposits suggest a very sharp permanent contact between ice sheet and tree populations during the late glacial period and the existence of one large refugial area, which may have contributed to the low level of geographic distribution of cpDNA diversity. There was no differentiation between the American and two gene pools (Gst=0.003). Furthermore, these results suggest that the introduced populations originate from a large sample of American populations.
BLACK LOCUST RESOURCES INVESTIGATIONS FOR DEGRADED AREAS REHABILITATION

Black locust (Robinia pseudoacacia L.), species native in Northern America, was imported in Europe around eighteen century. Because of its high rate of vegetative propagation it is wide spread everywhere. Among the fast growing deciduous trees species, R. pseudoacacia is the third used in forest stand establishment and artificial reforestations, after Eucalyptus and hybrid poplars, particularly in Eastern European Countries. The U.E. Project CT 98-135 "B.Lo.R.I.D.A.R.", just concluded, was developed to detect and characterize European R. pseudoacacia germplasm suitable for reforestation of degraded lands in unfavourable environments. This objective was be pursued by integrated researches at genetic, biochemical, eco-physiological level, completed by technological studies on wood characteristics as influenced by different environmental conditions. All these elements allowed to evaluate the possibility to spread Robinia stands in unfavourable environments in such a way to combine protective and landscaping functions together with an increase of the quality wood production. The project was carried out by complementary teams from 2 Eastern European Countries (Hungary and Bulgaria ) and 2 U.E. Countries (Greece and Italy) with Italian coordination. The main results obtained after three years of the project are: Molecular markers for European provenances identification; Molecular markers for characterisation of the open pollinated progenies of selected good performer clones/plus trees; Set of RAPD markers showing the largest weight in the poor and best performer discrimination. Clonal bank, including genotypes selected in each Country; Methodology for non-destructive (non-lethal) early evaluation of wood on standing trees; Characterisation of R. pseudoacacia juvenile wood; Set up of industrial parameters for transformation of black locust wood. This paper is mainly focused on the promising results obtained in the "genetic" sector. Because of it, the Authors warmly hope for the opportunity to continue the begun but not concluded researches in a new project including more Countries and research teams.
IS THERE PANMIXIS IN EXPERIMENTAL SEED ORCHARDS OF PRUNUS AVIUM L.?

Seed orchards are one of the way to obtain seeds for plantations in forests. However, the diversity of seeds which are finally used in plantations depends on: the effective genetic crossing (how many fathers contribute to pollination ?), the maternal proportions occurring in harvested seeds, and all hazards affecting seeds after the harvest. The inter-annual genetic stability of seed harvests remains unknown. Moreover, in the case of wild cherry (Prunus avium L.), the effective crossings among individuals that composed the seed orchard may also depend on the incompatibility groups existing in this species. In this study, we aimed at determining the factors that influence gene flow in three experimental seed orchards of Prunus avium L.: (i) an open pollinated one, (ii) a seed orchard in a greenhouse with all the individuals introduced at the same time and (iii) a second seed orchard in a greenhouse with late individuals introduced first, in order to synchronize flowering. The three seed orchards were constituted with the same 19 clones. About 20 seeds from each clone were analysed with microsatellite markers and a paternity analysis was performed. We tested whether the greenhouse seed orchards allow to limit genetic pollution in comparison with open pollinated seed orchards and whether the greenhouse seed orchard with synchronized flowering allow to increase panmixis among clones.
GENETIC DIVERSITY AND SILVICULTURE PRACTICES IN ITALIAN BEECH STANDS

A major challenge facing modern silviculture is to reconcile the traditional objectives of timber production with the demand for recreational and ecological functions of forest ecosystems which arises from the society. Most of this challenge regards the development and the refinement of close-to-nature silviculture systems, as they may allow environmental as well as productive aspects of forest management to be combined. There is increasing interest in maintaining biodiversity in our forest ecosystems and about the possible impact of forest practices and human activity on biodiversity. The preservation of the forest genetic pool is the basis of biodiversity, as it represents the basis for adaptation and survival of species and individuals, especially under changed environment and disease conditions. The utilisation of forests by man should take in account, probably more than in the past, of their biodiversity preservation as the forest populations are in the conditions to face and obviate climate changes (global change). The main objectives of this work were to study the relationship between the silviculture practices and genetic biodiversity on beech stands. The test sites of three pure beech stands were defined: i) two located in the Southern Italy (Campania and Basilicata), and ii) one located in the National park of Gran Sasso area (centre of Apennine chain). These stands have been characterised from vegetational, structural and silvicultural point of view. The Random Amplified Polymorphic DNA (RAPD) analysis was performed to evaluate the genetic characteristics. The Analysis of MOlecular Variance (AMOVA) and Spatial Genetic Analysis (SGS) were utilised to estimate the amount of genetic variability due to differences within and among stands, and the distribution of genotypes over the two dimensional space of a stand, respectively. The first analysis has given the information on the richness of genetic markers in each stands, indicating that genetic diversity may be to change as consequences of silviculture practices. The second one has permitted to know the genetic spatial distribution of mature trees and of their natural regeneration. Thus spatial structure is relevant to the measurement and management of the stand genetic composition.
Artificial regeneration is widely applied in the forest tree species. Many planted stands of European silver fir (Abies alba Mill.) show inferior health and stability in the adult age, however. This fact suggests that different seed sources used for stand establishment may have influenced their genetic structures if they are compared with natural populations. The objective of the present study was therefore:
- to analyze the effects of artificial stand establishment on the genetic structure, and compare them with
- temporal trends in genetic variations
GENETIC DIVERSITY AND (MICRO)GEOGRAPHICAL ISOLATION OF DUTCH JUNIPERUS COMMUNIS POPULATIONS

In the Netherlands, the dioecious species Juniperus communis L. (juniper) typically grows on heather fields and (former) sand dunes. During the past centuries, these habitats became fragmented or disappeared. Moreover, rejuvenation in Dutch J. communis populations is minimal. As a result, J. communis has become an endangered species. We recently started a study into the genetic diversity and relationships of the remaining populations. Based on historical distribution data, three main regions of occurrence were identified: Coastline, South-Limburg, and Central-Eastern Netherlands. These regions are characterized by specific soil types. The large majority of Dutch J. communis populations occur in the Central-Eastern region, and these were assigned to 9 microgeographical areas. The populations from these areas are isolated by wetlands, river valleys, or (former) peat bogs. For each of the regions and microgeographical areas, two (sub)populations of approximately 30 individuals will be sampled and examined using AFLP. Intra- and interpopulation genetic diversity will be established in order to determine the amount of genetic variation preserved, and to determine whether J. communis forms genetically distinct populations. The results will serve as a basis for programs to protect and restore Juniperus communis populations in the Netherlands, and for future international projects.
Pimpinella tirupatiensis is an endangered, narrow endemic medicinal plant with reputed medicinal values. Nutritional analysis of tubers of this species showed rich magnesium and phosphorous contents. However, increasing threat on it's population reduction coupled with limited number of populations necessiated immediate need for conservation of this rare species. RAPD analysis of existing populations revealed less genetic diversity within populations than the diversity observed at intrapopulation level. A population with relatively high genetic diversity with a percent polymorphism of 52.3% was chosen for in vitro studies. Micropropagation protocols were developed through, Axillary shoot proliferation, shoot tip culture, callus culture and through somatic embryogenesis. Methods were also optimized for in vitro tuberization and in vitro flowering in P.tirupatiensis. Encapsulated microshoots and somatic embryos were stored under low temperature and regenerated after a period of 90 days. The tissue culture raised plants were healthy and showed normal flowering pattern in all the methods standardized. The protocols developed were highly useful for largescale propagation and germplasm conservation of this rare unexploited member of Umbellifer for posterity.

In the FRAXIGEN project we are investigating genetic diversity and local adaptation in three European species of ash (Fraxinus angustifolia, F. excelsior, F. ornus), with the aim of providing guidance for the conservation, utilization and management of ash genetic resources. As part of this research, we are using neutral molecular markers (microsatellites in the nuclear and chloroplast genomes) to characterize population structure and patterns of mating and pollen flow, and to estimate the extent of inbreeding depression and local adaptation (in combination with networks of field trials) for each species. The species have different reproductive systems, and we are particularly interested in investigating partitioning of genetic diversity, gene flow, and differential fecundity among genders. Our poster describes the areas of the project in which we are using molecular tools, and details the laboratories and institutes involved in the research.
The flora of Finland includes two native elm species, wych elm (Ulmus glabra) and European white elm (Ulmus laevis). Wych elm occurs south of latitude 63 as less than 50 distinct populations and sporadic (individual) trees. European white elm has a fragmented 20x100 km distribution area consisting of ca. 2300 trees along a lake and river system in southern Finland and 2 populations outside this main area. Both species are protected by law and regarded as endangered on the basis of reduction of the population size and declining quality of the habitats. Allozyme studies indicate quite high genetic differentiation among the populations and for European white elm low heterozygosity compared to the average for deciduous trees. Adopted genetic conservation strategy for both elm species is ex situ graft collection. The collections are designed to serve both conservation and seed production. Presently, wych elm collection includes 168 clones from 38 stands and European white elm collection 83 clones from 19 stands. The collection will be planted as two identical replicates on separate locations, one of the replicates will be kept as a hedge in order to avoid DED as the disease might spread also to Finland in the future.
The red squirrel (Sciurus vulgaris) is the only squirrel species native to the UK. The advance of the grey squirrel is gradually confining the red squirrels to the large spruce dominated coniferous plantations in the north of England and to small, isolated fragments of woodlands. DNA techniques and museum collections have been used to determine genetic variation and population structure of red squirrels in the north of England over the last century. Genetic diversity can be relatively high in fragmented landscapes, so long as there is a sufficient network of "stepping stone" habitats. The implications of this evidence are far stretching for the conservation management of animal and plant species in fragmented landscapes such as found in Britain.
CONFORMITY ASSESSMENT OF EUROPEAN ELMS (ULMUS SP.) REGENERATED FROM LONG-TERM CRYOPRESERVED BUDS

Elm (Ulmus sp.) is a greatly appreciated species in demand for high quality, strong and ornamental timber. Genetic resources from the 3 indigenous European species (U. minor Mill., U. glabra Huds, and U. laevis Pall.) as well as from natural hybrids between U. minor and U. glabra are greatly endangered owing to recurrent Dutch elm disease during the last century, resulting in considerable reduction of adult elm populations. In France and Europe, in and ex situ conservation programs have been initiated in the past 15 years for the 3 species. However, it is largely unknown if such cutting-based programs in conjunction with natural vegetative (shoot, sucker) or sexual regeneration (seed) are sufficient to avoid genetic erosion of the Elm germplasm. More recently, AFOCEL has developed alternative, long term and efficient ex situ techniques of organ or tissue conservation in liquid nitrogen (cryopreservation) for several conifers and angiosperms, especially Ulmus species. At present, dormant buds of 226 clones have been cryopreserved in the frame of the EU RESGEN conservation program (CT 078-96) of elm genetic resources. Regeneration potential of dissected cryopreserved apices directly placed on culture medium is high for U. minor (39-100%), U. laevis (30-95%) and U. minor-glabra (52-73%). Interestingly, U. glabra apices only survived when micrografted on U. minor microcuttings (44-96% survival rate). Non cryopreserved (control cuttings) and cryopreserved material (cuttings and microcuttings) from 3 clones of U. minor and 3 clones of U. laevis were compared in field plantation test (randomized blocks of 5 plants) to assess quantitative traits and genetic conformity. Considering one phenological (flushing) and 5 morphological traits (height, collar diameter, number of branches longer than 10 cm, mean surface and length/width ratio of 3 leaves) after two vegetation years, no clear differences between cryopreserved and control plants could be detected. Significant differences observed for some clones were all in favour of cryopreserved plants. Molecular PCR investigation of genetic conformity were performed using 5 different primers (15-19 pb) designed to amplify variable number of tandem repeats (minisatellite regions). All clones could be distinguished using these markers. Similarity indices (0.92-1.00) based on presence/absence of fragment 500-2500 bp long with defined relative intensity strongly suggested molecular identity between cryopreserved and control plants.
CONSERVATION OF FOREST GENETIC RESOURCES IN SLOVENIA – OPERATIONAL ACTIONS

The forests cover more than 56% of the Slovenian territory. The forests are well preserved, 87% of them show a natural or close-to-natural species composition. With respect to the legislative basis and silvicultural practices the Slovenian forest are better preserved and sustainably managed than other middle European forests. The Forest Act (1993) preserves the forests as prescribed in the category VI of IUCN. Conservation of forest genetic resources is further based on the Forest Development Programme of Slovenia (1996), the Act on Conservation of Nature (1999), the Biological and Landscape Diversity in Slovenia – An overview (2001) with the Biodiversity Conservation Strategy of Slovenia (2001/2002). However, the questionable origin of some species reflects the problematics of the conservation of forest genetic resources (FGR) in Slovenia (i.e. Norway spruce & common oak); the species, which are rare (i.e. true & wild service tree) or at the border of their natural area of distribution (i.e. holm & false-cork oak), for which several cultivars can interact with the wild type (i.e. cherry, yew) or which numbers are reduced by forest pests and diseases (i.e. elms), are especially vulnerable and in a need for an active protection. Conservation of forest genetic resources is based on natural regeneration (in the case of autochtonous populations of forest trees) and regeneration of forests with planting and sawing of high quality forest reproductive material (FRM) of known origin, well adapted to the regenerated forest site. The operational level of conservation of FGR is based on mapping, research and active protection of supposedly autochtonous populations of forest tree species (within the network of forest gene reserves in situ), on flexible silvicultural planning, on the new Act on FRM (2002) with the regulations thereafter and on the development of Seed Storage and the Slovenian Forest Gene Bank (seed bank, study trials, living archives and other objects of conservation ex situ).
IMPLEMENTATION OF FOREST GENE CONSERVATION STRATEGIES IN SWITZERLAND

Successful conservation of forest gene resources requires a scientific basis which is obtained from a combination of ecological and genetic research. Additionally, the implementation of conservation efforts has to take into account silvicultural, technical, economical and social aspects. Of particular importance is the collaboration among different stake-holders, especially forest owners, forest managers, Cantonal and Federal administrators and researchers. In Switzerland, gene conservation concepts for several forest tree species are being developed on behalf of the Swiss Forest Agency in which research results (e.g. from genetic findings, distribution maps, forest history) are incorporated. In order to facilitate the step from research to implementation, both parts are merged in one single project: "Conservation and sustainable use of forest gene resources". The broad approach in the implementation of forest gene conservation in the framework of this project is presented with two examples: Oaks: In spite of the minor occurrence in the Swiss forests (2% of total growing stock, mainly Q. robur and Q. petraea), oaks are of great ecological and cultural value. In order to prevent a further decrease of oaks in Switzerland, the efforts for the conservation of gene resources are integrated in a holistic strategy for the promotion of the natural and cultural heritage of oaks in Switzerland. Silver fir: Silver fir (Abies alba Mill.) is one of the most frequent tree species in the Swiss forests (14.6% of growing stock) and of outstanding value in mountain forests. However, in the long term the species tends to decline owing to low recruitment, improper silvicultural methods and game damage. Therefore, the implementation of the gene conservation concept has to take into account all of these factors as well as the supply of well-adapted local reproductive material.
INTERNATIONAL NETWORK OF PROVENANCE TRIALS IN CORK OAK (QUERCUS SUBER L.)

Under the funding of European Union projects FAIR1 CT95 0202 and microaction B7 4100-as well as additional national funding, a network of 12 provenance trials and 6 progeny trials on cork oak (Quercus suber L.) was established during 1998 and 1999. The funds provided the means for harmonised selection of basic material representative for the natural range of the species and a common experimental design. The presentation focuses on the structure of the international network of provenance trials and its aims. Thirty-four provenances originating from Algeria (1), France (4), Italy (5), Morocco (6), Portugal (8), Spain (7) Portugal/Spain (1) and Tunisia (2) are represented. For each provenance 22 mother trees were sampled whose identity was kept along the process resulting in a total of 748 open pollinated single tree progenies. The twelve provenance trials were established in: France-1, Italy-3, Morocco-1, Portugal-3, Spain - 2, Tunisia-2. Number of provenances, selection criteria, experimental design, collection of material, raising of plants and early results are presented. Provenance trials enable the assessment of the total genetic variability and among population genetic variability of cork oak. They allow for gaining knowledge on the adaptation to local environmental conditions which supports the development of strategies for genetic improvement and conservation of genetic resources of the species. Early results on survival, growth and vigour are being used as the major parameters to assess adaptation of populations. In accordance recommendations on the use of provenance reproductive material will be provided. Results at age 2 to 5 (depending on the country maintaining the trial) reveal differences among provenances. Genotype by environment interactions are to be expected if the present patterns will proceed. Consequences for breeding and genetic improvement strategy and for conservation of the genetic resources of cork oak are discussed. Key words- Cork oak, Quercus suber, provenance trial, adaptive traits, breeding, genetic improvement, conservation of the genetic resources
The paper presents the results on the investigation on genetic structure of European black pine natural populations in the Republic of Macedonia and directions for conservation of its genetic resources. The gene diversity and differentiation were determined in six gene loci using the chloroplast simple sequence repeats (cpSSRs) markers. The level of haplotypic diversity (He) in the surveyed populations was ranging from 0.44 to 0.52. The proportion of genetic variation among the populations, estimated by the use of Rst was 0.024, thus indicating that about 2% of the variation is due to differences among populations, while 98% of the variation is due to the differences within populations. Gene flow (Nem) was calculated as 22.48 migrants per one generation, which represents very high value and shows that there is very high gene exchange among these populations. The genetically most differentiated population is (porece-POR), while the other three (Strumica-STR, mariovo-MAR and Berovo-BER) are genetically very similar. The phylogenetic relationship among the populations was determined with the cluster analyses. With regard to the conservation of Pinus nigra genetic resources in the Republic of Macedonia, a special attention should be paid to the (Porece-POR) population, because in this study it showed that in total, it has the highest number of alleles (25) with some of them being very rare with low frequencies.
CONSERVATION OF GENETIC RESOURCES OF BROADLEAVED FOREST TREE SPECIES IN UKRAINE

Results of activities carried out in Ukraine within the scope of the project «Genetic resources of broadleaved forest tree species in Southeastern Europe» funded by the Ministry of Finances of Luxembourg and coordinated by IPGRI are presented. A complementary approach was chosen encompassing the in situ conservation of genetic diversity of native forests, further development and use of technologies for ex situ conservation as well as genetically sustainable forest management. A comprehensive inventory of the existing system of genetic reserves and other in situ gene conservation units to start throughout the country, with an emphasis in 2001 on Quercus and Fagus resources. Particular emphasis was given to the inventory of rare broadleaved species in the relevant gene conservation units. All partners ensured the use of common information standards for description of the gene conservation units. The units inventoried were mapped using GPS to be associated to the relevant species distribution maps. In 2001, the inventory was carried out in 8 provinces: Ternopil, Chernivtsi, Zhytomyr, Rivne, Sumy, Kharkiv, Poltava and partly Crimea. 93 gene reserves, 11 «plus» stands and 416 «plus» trees have been inventoried. Totally 123 sample plots have been established. Results of the inventory are to be added to the existing EUFORGEN databases and to be made available on the Internet. The original national database containing more detailed information is under development. Feasibility studies on genetic inventories of beech (Fagus sp.) using RFLP and RAPD methods have been started. The map of natural distribution range of beech in Ukraine has been developed. The area covers two mountain regions in Ukraine: Carpathians and Crimea. In Carpathian region beech is represented by the species Fagus sylvatica. In Crimea beech is represented by Fagus taurica, which is considered as a subspecies of F. sylvatica or a hybrid between F. sylvatica and F. orientalis. Establishing clonal archive of the most endangered plus trees of Quercus robur L. using in vitro culture has been started. In order to support conservation strategies and propose options for further developing or updating legislation and policy systems, an analyse of the legal and policy framework in Ukraine of main legislative documents in force in the area of FGR conservation has been done.
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EUROPEAN Beech FOR FORESTATION AND DIVERSIFICATION: DEVELOPMENT OF FORESTATION TECHNIQUES AND ASSESSMENT OF THE GENETIC VARIATION IN REPRODUCTIVE MATERIAL

The cryo-storage of beech seeds at -196°C in liquid Nitrogen was successful. However, research and development is still necessary for storage and subsequent rearing of in vitro raised vegetative material. The method of raising plants in plug containers becomes more and more adequate the higher the level of qualification and improvement of the reproductive material is. By seeding instead of planting, much more cost-efficient reforestation methods for artificial rejuvenation of beech in coniferous forests may have been developed. The genetic variation in beech is maintained in the natural regeneration as well as in the artificial regeneration. The level of heterozygosity found in the mature stand and in the natural regeneration was found to be nearly identical (proportion of heterozygous genotypes). These results are very important for the choice of silvicultural treatments with respect to adaptability of beech stands. Some provenances avoid frosts by flushing their leaves late. They originate from regions with frequent late frosts. Afforestations in regions with late frost risks should be done with late flushing provenances. It can be concluded that it is possible to rank provenances for hardiness in spring with the electrolyte leakage method. However, since the correlation between leakage and the much more simple registration of phenology is high, there would be no relevance in using the leakage method in spring. It can be concluded that there are no indications that trees with false heartwood are less hardy than trees without false heartwood. Most beech provenances in Europe have re-migrated from the Balkan Peninsula, even into the Pyrenees. These results throw new light on the understanding of the genetic variation of beech populations, especially in this region. Until now it was assumed that in its southwestern distribution, beech originates from an Iberian refugium. This is obviously not the case. The ranking in economic returns was largely in agreement with the ranking in stem straightness. In tree improvement programmes stem form has to be given more weight. It will not be easy to find beech provenances that can be recommend for a large range of sites. Thus, specific, rather than general provenance recommendations are required. Among the 26 French provenances highly significant variation was found. This result has important implications for national provenance recommendations.
CREATING A NETWORK FOR SCANDINAVIAN FOREST GENE RESOURCE MANAGEMENT CO-OPERATION

Scandinavian countries - Denmark, Finland, Iceland, Norway and Sweden - co-operate on many sectors through joint Nordic organizations. Good experiences on Scandinavian cooperation on gene resources of agricultural plants and farm animals have created a need to fortify Nordic co-operation in the field of forest gene resource management. A description of creating a model for Nordic Forest Gene-network will be given. Organizing and network functions will be discussed.
ISOZYME AND MICROSATELLITE VARIATION IN A MANAGED AND UNMANAGED BEECH STAND IN THE NETHERLANDS

For beech a wide range of silvicultural regimes exists such as clear-cut, shelterwood, group selection, individual tree selection, coppice and coppice with standards. In The Netherlands clear-cut in combination with planting was most commonly practiced. Nowadays most natural beech forests are managed based on minimum intervention, while for the multifunctional forests a group-selection system is used. Different silvicultural regimes are likely to have different impacts on the genetic diversity and structure of the stands. We compared two Dutch beech stands ‘Pijpebrandje’ and ‘Solsebosje’ that differ in degree of management for their genetic diversity. The first one ‘Pijpebrandje’ is a semi-natural stand that has been extensively managed since the last century. The second stand ‘Solsebosje’ is an even-aged, regularly spaced monoculture. Both stands are located in an old forest area in the center of The Netherlands. The genetic diversity within and among the stands was studied using 12 isozyme and 4 microsatellite markers (Pastorelli et al, in preparation). Both stands were characterized by a high within-population diversity and did not differ significantly in their genetic diversity. The genetic differentiation between the stands was low (Fst of 0.014 for isozyme loci and 0.021 for microsatellite loci). Using the spatial autocorrelation procedure following Smouse and Peakall (1999) we found that a fine-scale genetic structure existed in the unmanaged stand (‘Pijpebrandje). Neighboring adult trees within the distance classes up to 30m. are genetically more similar. If this is due to a restricted gene flow by pollen and/or seeds in this stand should be further investigated. As expected, a random distribution of the genotypes was found in the planted stand ‘Solsebosje’. References R. Pastorelli, M.J.M. Smulders, W.P.C. van’t Westende, B. Vosman, R. Giannini, C. Vettori, G.G. Vendramin (2002) Characterisation of microsatellite markers in Fagus sylvatica L. and Fagus orientalis Lipsky. In preparation. Smouse P.E, R. Peakall (1999) Spatial autocorrelation analysis of individual multiallelic and multilocus genetic structure. Heredity 82: 561-573.
GENETIC VARIATION IN THE ENDANGERED WILD APPLE (MALUS SYLVESTRIS (L.) MILL.) IN BELGIUM AS REVEALED BY AFLP AND MICROSATELLITE MARKERS. CONSEQUENCES FOR CONSERVATION.

The genetic variation within and between wild apple samples (Malus sylvestris) and cultivated apple trees was investigated with AFLP and microsatellite markers in order to develop a conservation genetics program for the endangered wild apple in Belgium. In total 76 putative wild apples (originating from Belgium and Germany), 6 presumed hybrids and 39 cultivars were typed at 12 SSR and 139 AFLP loci. Principal coordinate analysis and a model-based clustering method classified the apples into three major gene pools: wild Malus sylvestris genotypes, edible cultivars and ornamental cultivars. All presumed hybrids and two individuals (one Belgian, one German) sampled as M. sylvestris were assigned completely to the edible cultivar gene pool, revealing that cultivated genotypes are present in the wild. However, gene flow between wild and cultivated gene pools is shown to be almost absent, with only three genotypes that showed evidence of admixture between the wild and edible cultivar gene pools. Wild apples sampled in Belgium and Germany constitute gene pools clearly differentiated from cultivars and although some geographical pattern of genetic differentiation among wild apple populations exists, most variation is concentrated within samples. Very concordant conclusions were obtained from AFLP and SSR markers, which showed highly significant correlations in both among genotypes and among samples genetic distances. The results are discussed in the context of a conservation program for this endangered species.
Dalbergia sissoo, known as Indian rose wood, is a multipurpose leguminous tree species. Its natural distribution is mainly in the South-Asian region (Nepal, India, Pakistan and Afghanistan). It has also been introduced to many other parts of the world mainly in tropical countries as an exotic species. The species is used for different purposes such as furniture, plywood, fodder, shade tree for streets and backyards, fuel wood and for some folk medicines. In Nepal, Dalbergia sissoo is distributed throughout the Terai (plain) region, mainly in plantations. About 40% of all forest trees in Nepalese plantations are D. sissoo. Natural stands are close to extinction due to high population pressure by local people. However, a few natural relic stands are remaining. Recently, forest management of the species has been challenged by the outbreak of a die-back disease and the formation of poorly formed stems. The introduction of genetically inferior planting materials (seeds, stumps, root suckers) from unknown sources is believed to be one of the possible causes of these problems. We conducted a genetic inventory of the species in order to get information on patterns of genetic variation within and among populations in Nepal. Isozyme and cp-DNA analysis supported the hypothesis that natural stands and plantations are of different origin. Our preliminary study suggested that gene flow between natural stands and plantations is rare.
THEORY & PROBLEMS IN CONSERVATION OF FOREST GENETIC RESOURCES IN THE SLOVENIAN FORESTS

The forests cover more than 56% of the Slovenian territory. The forests are well preserved, 87% of them show a natural or close-to-natural species composition. With respect to the legislative basis and silvicultural practices the Slovenian forest are better preserved and sustainably managed than other middle European forests. The Forest Act preserves the forests as prescribed in the category VI of IUCN. However, the questionable origin of some species reflects the problematics of the conservation of forest genetic resources (FGR) in Slovenia: Norway spruce was in the past moved around the Austro-Hungarian Imperium with no traces of its origin; common oak in the virgin forest reserve Krakovski gozd is of questionable origin; the species, which are rare (i.e. true & wild service tree)or at the border of their natural area of distribution (i.e. holm & false-cork oak), for which several cultivars can interact with the wild type (i.e. cherry, yew) or which numbers are reduced by forest pests and diseases (i.e. elms), are especially vulnerable and in a need for an active protection. Conservation of forest genetic resources is based on natural regeneration (in the case of autochtonous populations of forest trees) and regeneration of forests with planting and sawing of high quality forest reproductive material (FRM) of known origin, well adapted to the regenerated forest site. The operational level of conservation of FGR is based on mapping, research and active protection of supposedly autochtonous populations of forest tree species (within the network of forest gene reserves in situ), on flexible silvicultural planning, on the new Act on FRM with the regulations thereafter and on the development of Seed Storage and the Slovenian Forest Gene Bank (seed bank, study trials, living archives and other objects of conservation ex situ). The FRM can be used only within the same provenance regions and elevational zones. The seed should derive only from selected seed stands with an efficient effective population size and an efficient number of seed trees. The main problems are small quantities of seed needed for the Slovenian forests (and therefore the negative ratio between the costs for production and available budget), the permanent cuts for the forestry and forest seeds and nurseries sector and under-developed infrastructure for long-term conservation of forest seeds.
MODELING FOREST GENETICS, -ECOLOGY AND -MANAGEMENT: OUTLINE OF THE FOR-GEM MODEL

In the DynaBeech-project it is aimed to assess the impacts of previous silvicultural regimes on genetic and ecophysiological diversity within beech forests in Europe and to provide guidelines for both the optimisation of the adaptive potential and the in situ conservation of diversity of beech forests through appropriate silvicultural regimes. The genetic and ecophysiological information gathered by experiments and field observations will be integrated in the FOR-GEM model that includes a phenological submodel, a genetic submodel considering recombination and segregation, a gene flow sub model based on dispersal of pollen and seed, a general spatially explicit forest growth sub model and subsequently a submodel to evaluate management regimes with respect to their long-term effect on forest functioning and structure. An outline of the model assumptions, processes an outcomes is presented.
Traditional forest management of European beech forests consists of large-scale felling with few remaining trees to produce the next generation. This may have significantly disturbed the genetic and ecophysiological diversity. In general, high diversity is assumed to be very important for the adjustment to environmental changes. DynaBeech plans to assess the impacts of previous silvicultural regimes on genetic and ecophysiological diversity within beech forests in Europe and to provide guidelines for both the optimisation of the adaptive potential and the in situ conservation of diversity of beech forests through appropriate silvicultural regimes. DynaBeech aims are: 1) providing tools for evaluating biodiversity, 2) understanding the functioning and diversity of forest ecosystems and 3) assessing the interactions with forestry activities. To achieve the objectives, the project uses a multidisciplinary approach by integrating state-of-the art knowledge on genetics, ecophysiology and spatial explicit simulation modelling. A pairwise comparison of managed and unmanaged study plots will be performed at 5 forest sites in Europe. In these plots the genetic diversity will be assessed based on biochemical and molecular markers. At two sites gene flow (pollen and seed dispersal) will be studied through a parentage analysis. For a better understanding of the functional role of diversity, heritabilities of some functional traits will be estimated and molecular markers related to functional traits (QTL mapping) will be identified. For this purpose, material already available at some participants will be used. Simultaneously, the ecological variation will be assessed by observation of the trees in the plots and by studying the characteristics of seedlings derived from the plots in nursery experiments. Phenological (e.g. bud burst), ecophysiological (e.g. photosynthesis, stomatal conductance) and morphological (e.g. specific leaf area) characteristics will be studied. The genetic and ecophysiological data will be integrated in different models: a phenological model, a genetic model including recombination and segregation, a gene flow model based on dispersal of pollen and seed, a general spatially explicit forest growth model and subsequently a model to evaluate management regimes with respect to their long-term effect on forest functioning and structure. End-users, working in the field of seed improvement, forestry and nature management will actively participate in the project. They will provide relevant silvicultural scenarios, which will be evaluated by modelling. Finally, scientists and end-users will together formulate general guidelines and recommendations for management based on the modelling results and evaluate the implications of these guidelines for practical forest management. Relevant milestones and expected results for the project are: isolation of microsatellites to be used for the genetic diversity and gene flow study, data set of genetic parameters available for model, formulation of management scenarios by end-users, all ecophysiological parameters available for model, model evaluation of all forest management scenarios, evaluation of modelling results, final guidelines for management.
CONSERVATION OF PEDUNCULATE OAK (QUERCUS ROBUR L.) IN YUGOSLAVIA

The forests of pedunculate oak (Quercus robur L.) are economically one of the most valuable forests in Europe. In Yugoslavia they occur naturally in the valley of the river Sava, and less abundantly in the valleys of the rivers Danube and Morava. The in situ conservation started by selecting the seed stands in the populations of Ravni Srem (Sava river). The ex situ conservation of this species in Yugoslavia started by selecting the phenotypically superior genotypes in seed stands and other natural populations. The main criteria for the selection were the straightness of stem, branching, fast growth and resistance to mildew (Microsphaera alphitoides). They were used in the establishment of the clonal seed orchard on 7 ha at the locality "Banov Brod". The seed orchard was established from 86 genotypes, which were then "multiplied" by grafting into 2520 ramets on the area of 7 ha at the site "Banov Brod" (Vojvodina). Depending on the scion thickness, five techniques of grafting were applied, e.g.: simple copulation, English copulation, "mjesok" - little sack, cutting, and "goat leg". The seed orchard is composed of all 4 varieties indigenous in the valley of the river Sava. They are early pedunculate oak (Q. robur var. praecox), typical pedunculate oak (Q. robur var. typica) and two varieties of late pedunculate oak (Q. robur var. tardiflora and Q. robur var. tardissima.)
PINUS RADIATA GENETIC RESOURCES FOR WOOD QUALITY TRAITS - CELLULOSE CONTENT VARIATION AND MOLECULAR DIVERSITY OF CANDIDATE GENES FOR THE CELLULOSE BIOSYNTHETIC PATHWAY

Pinus radiata (D.Don) is the premiere softwood plantation species in Australia and New Zealand. Current wood producing and breeding populations are largely derived from two provenances, Monterey and Ano Nuevo, among the five original provenances of the species. These two in situ provenances occupy less than a few thousand hectares and Pinus radiata is currently listed as threatened in the USA due to urban development, disease and agricultural activity. The five in situ stands along with the small areas of provenance trials and gene conservation plantings in Australia and New Zealand (less than 1000 hectares) contain far more genetic diversity than all the plantation areas combined. Assessing, accessing and utilising this diversity is becoming a major challenge for tree breeders and plantation managers as many desirable alleles are likely to be contained within these small populations, especially for newly desired selection criteria. Molecular methods offer a number of new avenues for achieving these aims, but their application has to be adapted to pine species with constraints including long generation times and large genome size. We present a project that aims 1) at assessing the sequence polymorphism of a few selected candidate loci of the cellulose biosynthetic pathway, and 2) at analysing their association with cellulose content phenotypic variation. The material comes from one of the two provenance-progeny trials planted in 1980 in Batlow, New South Wales, Australia, that was based on a collection of the original provenances made in 1978 by CSIRO. Cellulose content is being determined gravimetrically for two annual rings for each of 470 individuals from 234 families from all five provenances. Results on cellulose content heritability and variation between provenances will be presented, as well as molecular variation of sucrose synthase and cellulose synthase gene family members that is being investigated using both SSCP markers and direct sequencing. This project brings new insight into the analysis of genetic variability at selected loci and the testing of methods for the dissection of complex traits.
The present study has been conducted to investigate (i) the potential of the existing black poplars along the Dutch-Belgian Meuse to act as a source population for recolonisation of the floodplains (ii) gene flow between the cultivated poplars and the native P. nigra and (iii) whether introgressed seedlings can colonise the riverbanks of the Dutch-Belgian Meuse. The study area is a nature conservation area of 3000 ha where the main objective is the restoration of river dynamics and the riparian ecosystem of the Dutch-Belgian Meuse over a distance of 50 kms. The genetic and phenological diversity of the remaining black poplar trees on the banks of the Dutch-Belgian Meuse was investigated by using AFLP and microsatellite markers and by observing the flower biology. Sixty-four seeds were collected from 2 open pollinated female black poplar trees and 33 seedlings that spontaneously colonised the river banks, were sampled. The genetic origin of the open pollinated progenies and of the Populus seedlings from the riverbanks was studied by using morphological characteristics, isozymes, the diagnostic locus win3, AFLP and microsatellite markers. The genetic diversity of the relicts was too small to act as a source for recolonisation along the Dutch-Belgian Meuse (only 4 different genotypes were found!). Although this is a limited study, the result show that a high level of potential introgression can occur. This is in contrast with other studies were low levels of introgression (0 to 5%) in Black poplar were detected. Seedlings of the riverbanks were originating from spontaneous hybridization within P. x euramericana and between P. nigra and P. x euramerica. Introgressed seedlings seemed to be well adapted as they survived the river dynamics over several years. P. nigra cv. italic was a potential father for only one seedling. No seedlings of pure black poplar were found on the riverbanks. Based on these results and preliminary results of ongoing research on pollen competition between conspecific and heterospecific pollen, guidelines for minimising introgression and for the restoration of Black poplar along the Meuse are given.
EVALUATION OF GENETIC DIVERSITY IN EUROPEAN WILD AND CULTIVATED GENOTYPES OF PRUNUS AVIUM AND SOME RELATED SPECIES OF THE SUBGENUS CERASUS USING CPDNA MARKERS

The genus Prunus is a wide one including from 77 species to 150 (depending on the authors) divided into five subgenera. The subgenus Cerasus includes the cultivated cherry trees (sweet and sour cherry) and their cherry related species. The objective is to study the chloroplast DNA variation of those species in order to establish phylogenetic relationships between them. This diversity has been analysed by PCR-RFLP. Eight couples of primers - enzymes were used for this analyse. The sampling include a large sample of wild and cultivated genotypes of Prunus avium species spread all over Europe and smaller samples of Prunus cerasus and few other species. This study take part in a larger programme analysing the genetic diversity of cultivated and wild cherry trees (Prunus avium) with nuclear and chloroplastic markers. Hypothesis on the domestication of cherry trees will be proposed. Relationships between P. avium, P. cerasus and some other species in the subgenus Cerasus will be discussed. The results will also provide a large knowledge of the chloroplastic diversity and will help us in the management and conservation of genetic resources of Prunus.
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