

GENETIC RESPONSE OF FOREST SYSTEMS TO CHANGING ENVIRONMENTAL CONDITIONS – ANALYSIS AND MANAGEMENT

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Preface

Forest ecosystems and various systems of plantations are increasingly subjected to substantially changing environmental conditions and corresponding modifications of abiotic and biotic stress components. Interdisciplinary efforts are required in order to jointly meet the challenges and the demands of new and complex stress constellations. Scientists are requested to contribute efficiently to sustainable development and to work out solutions for problems which are ready for transfer into practical management. This is the reason why this conference was subtitled "Analysis and Management".

The IUFRO Conference in Freising/Munich followed the IUFRO Conference in Vancouver 1996 and was linked to the scientific processes which were initiated during the IUFRO-Conferences in Beijing and Edinburgh 1998. In particular, one of the major objectives of this conference was to substantially enhance co-operation between the IUFRO-Divisions 2 and 7 and to continue the progress of bridging gaps between forest genetics and related research fields.

This conference was attended by more than 90 scientists from 23 countries in Europe, Asia, North America and South America. It was sub-structured into eight half-day sessions. In addition, two poster sessions and small satellite meetings helped in the communication and discussion of results. A field trip to high altitude forests in the Bavarian Alps addressed impacts of changing environmental conditions on vulnerable forest ecosystems focussing on forest genetics, growth and silviculture.

The results of the eight sessions are surveyed in the subsequent chapters. The respective chairmen and rapporteurs were K. Percy, F. Scholz / B. Degen, D. Karnosky, X. Shen, H. Hattemer, G. Vendramin, L. Paule, and A. Kremer. Revised and accepted contributions are scheduled to be published in 2001 (Kluwer Academic Publishers).

Session reports

Interdisciplinary (Session 1)

Papers presented new data at three different scales of forest genetic response to environmental stress. A. Menzel pre-

sented on phenology as a bio-indicator for increasing air temperature. Changes in temperature (climate change), particularly in the spring, may be reflected in timing of phenological processes such as flushing. Using 1959–1996 data from the phenological network of the German Weather Service, and observational data from the European-wide International Phenological Gardens, linear trends were calculated. Major shifts were noted including: spring phenology (bud break) was advanced by 6 days and autumn events (fall coloration) were delayed by 4.8 days. This has resulted in a lengthening of the growing season by 10.8 days since the early 1960's. Although data indicated the growing season in Central Europe could be further lengthened without incurring further risk from late spring frost, implications of interactions with increasing frequency of extreme events, particularly in winter, is unresolved and may be critical in forest response.

Simulations of growth dynamics of mixed mountain forests under changing climatic conditions were presented by Biber and Pretzsch. Using the distance-dependant, climate-sensitive forest growth simulator SILVA, simulation runs were made following calibration of the model with data from spruce, fir, and beech mixed-forest research plots in the Werdenfels Mountains, Bavaria. Stand development was predicted for recent CO₂ atmospheric concentrations and doubled CO₂. Under the 2xCO₂ scenario, beech vitality increased while those of spruce and fir did not change significantly.

Discussion focussed on parameterisation of the model. Ozone is a significant stressor of vitality in these forests and may predispose them to insects and disease. Genetic considerations suggested for parameterisation were some measure of stand origin (provenance) and phenotypic response to 2xCO₂. As models assume random risk, it was felt that heritability responses in growth could be used to breakdown phenotypic variability.

International collaboration in conservation and sustainable use of forest genetic resources was addressed by v. Breugel and Turok (IPGRI). An important step in securing long-term commitment is to agree on priority species for gene conservation programs using three criteria: (1) threats imposed on the species (i.e. forest practices, air pollution,

Breugel and Turok (IPGRI). An important step in securing long-term commitment is to agree on priority species for gene conservation programs using three criteria: (1) threats imposed on the species (i.e. forest practices, air pollution, climate change, decreasing population sizes); (2) value attributed to the use of the resource; and, (3) representativeness. Establishment of the European Forest Genetic Resources Program (EUFORGEN) was highlighted as an example. The most important output from such networks is the development of regional conservation strategies. Participants strongly stated that commitment must begin with the national organisations if networks are to succeed. It was agreed that North American networks should be linked to those in Europe for a global perspective.

During the podium discussion of Session 1, it is concluded that climate change is clearly indicated by phenomena such as prolongation of the growing season in Central Europe, the reduction of glaciers in the European Alps, the melting of the ice caps of the poles and the increase of the level and the temperature of oceans. Impacts on forest ecosystems were discussed by mainly addressing the provenance level and possible threats caused by modifications of the biotic environmental conditions. Concerning yield science and related modelling, the necessity of an integration of genetic parameters was pointed clearly.

Genetic response to stress (Session 2)

The presentations considered different kinds of abiotic and biotic inferences following air pollution, climate change, insect pests and "reproductive stress". The papers described the impact of these stress factors on mating systems and on selection. Different strategies were communicated for rehabilitation of forests damaged by stress. Various tests on adaptation of provenances, families and clones to specific stress factors presented during Session 2 were directed towards the selection of appropriate material.

Mating system

As a result of the reduction of population size and population density, "Reproductive stress" leads to reduction of mating contacts, increase of self-pollination and enforced mating preferences for neighbour trees (Hattemer *et al.*). Reduction of population size and density could be caused either by increased mortality due to air pollution or forest management practices. The negative influence of pollution on reproductive organs was demonstrated by Tretyakova (abstract, no presentation). Fertility can be reduced by air pollution. *Abies* and *Larix* species were found to reveal different reproductive organ sensitivities to pollution. In addition, intra-specific genetic variation of tolerance was verified.

Viability / selection

Selection and vegetative propagation of SO₂-resistant clones of *Picea abies* and their response in six trial plots in SO₂ polluted environments were reported by Wolf. In terms of survival rate, height growth and stem forms, significant differences between the SO₂-resistant clones and other refer-

ence material were found. SO₂-resistant clones did not show superiority for all tested traits. Hence, the study indicated difficulties in the transfer of results from SO₂-resistance breeding to forest management. Liesebach *et al.* presented results of the IUFRO Norway spruce provenance experiments 1964/68. Whereas provenances from a few regions showed appeared adapted at all sites, most of the provenances performed well only under specific climate conditions.

Percy *et al.* analysed the physiochemical characteristics and properties of three aspen clones growing along an ozone gradient through Wisconsin – Michigan, USA. They found that ozone significantly modified chemical composition of the developing wax layer which varied significantly between sites, clones and leaf surface.

Within a screening programme for genetic resistance of white pine weevils in British Columbia, Canada, Alfaro *et al.* identified resistant families able to withstand very high weevil population density. The impact of selection was indicated by the fact that those parents from locations with high weevil hazard yielded higher proportions of resistant trees.

Several authors presented studies on possible strategies for rehabilitation of forests damaged by environmental stress. Kormutak *et al.* showed results of inter-specific hybridisation experiments with Mediterranean, North American and Asian fir species. The aim of these experiments was to produce hybrids with increasing tolerance to air pollution. Artificial hybrids were produced mainly within the Mediterranean fir species. Some hybrids showed heterosis effects regarding growth rates or emission pollution tolerance. Results of reforestation experiments in Poland with the two alder species, speckled alder, *Alnus glutinosa*, and black alder, *Alnus incana*, were presented by Mejnartowicz. In an area with heavy metal-contaminated soils, near in the vicinity of a copper smelter, intra-specific and inter-specific differences in sensitivity to pollution were found.

The discussion concluded that investigations on genetic response to stress offer the chance to integrate ecology and genetics. But, to improve understanding of forest ecosystems there is still a missing link between tests of provenances and clones on the one side and population genetic studies on the other. On the other hand, we observe changes of allele and genotype frequencies at marker loci due to specific population genetic processes but in most cases we do not know the relevance of marker gene loci to specific stress factors. Micro-scale mapping of QTL is an approach to fill this gap.

Genetic markers as bioindicators (Session 3)

This session comprised five presentations on various types of genetic markers used to detect biological stresses, e.g. terpene synthase genes as indicators of white pine weevil resistance in spruce (Wang *et al.*), or DNA marker fragments as indicators of *Phytophthora* pathogenicity in penduculate oak and European beech (Schubert *et al.*) and abiotic stresses: QTL's and PQL's for detecting differences in drought tolerance in maritime pines (Plomion *et al.*, presented by A. Kremer), O-methyltransferase activity as a marker of ozone stress in Scots pine (Ernst *et al.*), and PCR-based DNA markers for examining sensitivity of Norway

Following the six formal presentations, there was a discussion about whether or not the loss of genetic variability in response to stress had been proven. D. Karnosky provided published evidence for the loss of sensitive *P. strobus* genotypes in northeastern US forests exposed to elevated O₃ concentrations since the 1970's. It was generally agreed that reduction in diversity in the gene pool of sensitive species may in fact be more widespread than currently documented. K. Percy strongly supported the enhanced development of biochemical/genetic markers and their adoption in international forest health monitoring. State of science reviews on air pollutant-forest case histories have recently revealed fundamental changes to physiological/ biogeochemical processes induced by air pollution, despite lack of corroborative evidence from national monitoring networks. Sensitive, quantitative indicators with defined dose-response relationships are urgently required to scientifically assess forest health.

Participants highly recommended that genetic markers as bioindicators (Müller-Starck and Schubert) be promoted amongst ecologists and forest managers as possible quantifiable techniques for understanding stress impacts on genetic variability as a component of biodiversity. Current problems with bioindication accrue from the fact that cause-effect relationships are complex, dose-response is not linear, indicator(s) may not be responsive to the stress(s), measured, time-scales of response vary and various levels of bioindication can be measured from organelle all the way up to stand. Molecular markers appear to be ready for application in bioindication with inclusion of the monitoring of pathogens in tree populations (various tissues, soils) and in forest reproductive material.

Geographic variation (Session 4)

The main focus of this session was the study of causes and consequences of genetic variation, including geographic variation within species and genetic differentiation among species:

The comparison of quantitative traits (germination, height growth, phenology), and genetic characters (isoenzymes, RAPDs, cpSSRs) revealed corresponding patterns of geographic variation with respect to glacial refugia in case of *Pinus halepensis* (Alia *et al.*). Based on isoenzyme gene markers, differences in gene diversity were verified for different geographical regions of *Pinus pinaster* (González-Martínez *et al.*). These follow clinal patterns. For *Pinus cembra*, very low diversities and heterozygosities were monitored throughout the Alpine Mountains using isoenzyme gene markers (Klump). Evidently, genetic bottlenecks can be expected as a consequence of intensive utilisation by man. Differences in the phenology of populations of *Picea abies* were studied in connection with genetic variation at isoenzyme gene loci (Prus-Glowacki *et al.*). Intensive gene flow between populations from high and low altitudes in Southern Poland appears to compensate genetic differentiation with respect to the marker loci.

Clear genetic differentiation was verified between populations of *Araucaria angustifolia* in Northern and Southern Brazil (Sousa and Hattemer) via isoenzyme gene markers. Results provide very useful criteria for gene conservation. Based on cpDNA haplotypes, strong genetic differentiation within and between regions north of the Alps and the Da-

nube valley was demonstrated for European white oaks (Csaikl and König, presented by König). Maternally inherited markers appear as useful provenance markers and help to understand the re-colonisation pathways of white oaks. A study on taxonomy and phylogenetic relationships within European and Eastern beeches (*Fagus sylvatica*, *F. orientalis*) by means of isoenzyme gene markers revealed four genetically differentiated regions along Grand Caucasus, Western Asia Minor, and South Eastern Europe (Paule and Gömöry). There is evidence for transitional taxa (*F. moesiaca*, *F. taurica*).

New views at the concept of autochthony and of adaptation processes were presented in light of changing environmental conditions (Schoppa and Gregorius). During the discussion, both, support and disagreement with the views presented were evident.

During the general discussion it was pointed out that autochthony does not necessarily mean that the respective populations are optimally adapted. Furthermore, the effectiveness of gene flow and the limitations of the indicative value of maternally inherited haplotypes were discussed. For the purpose of gene conservation with respect to different species at various regions, the verification of the dynamics of genetic variation under global environmental change and the integration of such knowledge in multiple management activities is of a great importance. Priority should be given to combining the results achieved by using traditional methods with new genetic marker technology.

Genetic resources (Session 5)

Genetic conservation measures are indispensable in view of the significant human impact on forest tree populations, such as logging (Rajora and Mosseler), silvicultural management regimes (Konnert and Hussendoerfer), air pollution, certain breeding methods, and others. Research is needed in order to create a solid basis for conservation measures. The theory underlying the sustainable management of genetic resources was explained by Gregorius (see topic "Concepts and scenarios (Session 8)"). The following five questions appear to be most important.

(1) What is present? The answer to this question requires the analysis of populations with appropriate markers. Several authors presented results of various scales (Tolun *et al.*, Muratorio *et al.*). Isoenzyme gene markers have helped us gain most knowledge on the genetics of tree populations. DNA markers are said to be more efficient, although there is no such thing as general superiority in information content. Nevertheless, certain types of DNA markers have proven to be more informative for the study of descent and of random genetic processes. Several authors (Sagnard and Fady) have stressed the need for measuring adaptive phenotypic traits that are under strict genetic control.

(2) Findings on genetic markers must be interpreted in the light of ecology and evolution (including history). The large number of species requires much effort in adaptation and development of laboratory methods. However, markers which are proven adaptive under the study conditions may not help to identify those populations which may be most adaptable in an uncertain future. Therefore, findings on adaptive, genetically controlled phenotypic traits must be utilized (Sagnard and Fady). Only a few authors (Muratorio *et al.*) reported on globally and locally rare species which

typically occur in mixed stands. They have raised the most challenging problem in genetic conservation.

(3) The methods for the conservation of endangered species in their ecosystems or at least in their respective forest communities must be developed. A precondition for successful conservation and regeneration of resources is knowledge of the reproduction systems of both target and non-target species. All functions of their genetic systems must be maintained. Particularly the reproduction system with its central importance for the transmission of genetic information from one generation to the next must work. This is critical for the possibly scattered populations of the rare target species. Wang and Hattemer reported on gene flow within and among pure stands, Krabel *et al.* on reproduction under various environmental conditions. The development of methods may include techniques for enrichment by locally rare or absent genetic variants, as was discussed by Müller-Starck during the field excursion.

(4) The complexity of reproductive systems leads to spatial heterogeneity of tree populations and their seed production. This leads to enormous problems in drawing representative samples from the seed produced by a stand. Gillet showed that conventional sample sizes may be far from what can be called appropriate. In addition to adequate sample size, the design of sampling is of crucial importance for the validity of comparisons between populations and the far-reaching decisions based on them. Lexer *et al.* reported on methods for efficient plausibility checks of single-tree seed lots.

(5) The conservation of genetic resources can be performed only with close cooperation between geneticists and silviculturists. There is a need for the transfer of genetic results and genetic insight. We must improve awareness of genetics into silviculture.

Last, but not least, it has to be stated that concentrating conservation efforts on gene reserves may be risky. We may lose resources by fire, local human population pressure, or because of high-priority building operations.

New tools in forest genetics (Session 6)

In recent years, great efforts have been made to identify highly efficient methods for screening genetic diversity and for mapping in forest tree species. Many efficient markers, were identified not only in the nuclear genome but also in the chloroplast and mitochondrial genomes, which are uniparentally inherited and therefore transmitted by pollen and/or seeds. Markers were identified in both coding and non-coding regions and used for many different purposes, in particular for (a) genetic linkage mapping in conifers; (b) genetic diversity analysis in plants; (c) spatial genetic analysis; (d) gene flow studies.

Molecular analyses were also performed to understand the structure and organisation of a conifer species characterised by a very large genome, like *Picea abies*. An advanced mapping project was presented with *Picea abies*, the main objective being to produce a highly saturated linkage map (Scotti *et al.*). A combination of different strategies was followed in order to get an even coverage of a very large genome and to identify both, random unexpressed regions and gene related portions of the chromosomes (hyper variable nuclear SSRs, multi-locus AFLPs, sequence-specific S-SAPs and gene-based ESTs).

Because of the high degree of conservation of the chloroplast genome, many "universal" markers were used to study the geographic distribution of gene diversity in many different species including trees. As compared to nuclear markers, organelle markers partition a larger amount of diversity among populations. Mode of inheritance and life-history attributes are predictors of the differentiation level detected with these markers in various plant species (Fineschi *et al.*). Many recently performed studies shed light on phylogeography and post-glacial re-colonisation processes in forest species.

Highly polymorphic mitochondrial (maternally inherited) markers were identified in *Picea abies* (Gugerli *et al.*) and used to analyse the distribution of diversity among alpine populations. Maternally inherited markers best resolve migration pathways, because of their transmission through seeds and not through pollen. The origin of variation of the mitochondrial marker is linked with the presence of a minisatellite region in the *nad1* intron2. A decrease of variation was observed from eastern to western populations and a clear congruence between palynological and molecular data was observed.

Nuclear microsatellite markers were used to analyse Alpine populations of *Picea abies* (Magni *et al.*). This markers showed a very high level of variation within populations and revealed a significant deficiency of heterozygotes in all populations. Hypervariable chloroplast microsatellite markers were also identified and sequence characterised in *Abies alba* (Ziegenhagen *et al.*). Investigations at two polymorphic microsatellite loci exhibited a distinct geographical pattern of genetic variation through the natural range of this species. Due to the high degree of variation of these markers in this species, paternity analysis was efficiently performed.

PCR/RFLP of introns of nuclear genes (peroxidases, phenylalanine ammonia lyase, S-RANases, cinnamoyl alcohol dehydrogenase) was proposed as an approach to detect polymorphism in some broadleaved species (B. Heinze). In *Populus*, the analyses of these regions allowed the detection of introgression of genes from hybrid clones into native *Populus nigra* seedlings. In *Prunus avium*, information about the mating system of this insect-pollinated, self-incompatible species was obtained.

The transfer of microsatellite markers developed in one species to others is an efficient tool used to reduce the costs of marker development. Nuclear microsatellites developed and characterised in *Populus tremuloides* were successful in PCR amplifying genomic DNA and in resolving microsatellites of comparable sizes in other 24 *Populus* species, three of their interspecific hybrids and two *Salix* species (Rajora *et al.*). 38 *Populus tremuloides* individuals were uniquely fingerprinted based on their genotypes at only five microsatellite loci.

In order to understand the structure of Norway spruce genome, 120 clones corresponding to highly repetitive sequences, were isolated and sequenced (Zuccolo *et al.*). Long interspersed repeats seem to form a very large proportion of repetitive DNA in Norway spruce, while tandem repeats (satellite and microsatellite) do not seem to appear abundantly. A remarkable feature of conifer genomes is the high degree of suppression of CG dinucleotide both in repetitive and single copy sequences, like in mammalian genomes. This feature clearly distinguishes the conifer genomes from

those of angiosperms.

Generally, maternally inherited markers reveal significant information on the phylogeography, while paternally inherited markers such as chloroplast microsatellites in conifers showed a great efficiency for monitoring gene flow, performing paternity analysis and tracing past genetic processes like drift or isolation.

A high degree of “universality” of chloroplast markers allows the transfer of primers from one species to others belonging to different taxonomic classifications. The efficiency of screening molecular markers can be increased considerably through the automation of the procedures (e.g. multiplex of PCR reaction, multiple loading of a single gel, automated sequencing, robotic workstation etc.). Despite progress during the last years, many questions are still open, which can be summarised in a simple statement: “Which marker for which purpose?”.

Quantitative genetics and tree breeding (Session 7)

Within this session the main attention has been paid to: (1) to breeding programmes including also adaptive traits, (2) mating system estimations in natural and breeding populations, (3) breeding using molecular markers and QTL's, and (4) genetic monitoring and certification of seed samples.

Hamann *et al.* presented a paper aimed at the breeding programme of *Alnus rubra* including phenotypical traits and growth rates. They compared breeding populations with existing climatic conditions and the scenario of climatic changes. Shen and Chen presented results of a broader provenance experiment with *Platycladus orientalis* with the aim to test controlled drought stress and frost resistance. Hertel and Paul presented the study aimed at the resistance breeding in *Picea abies*. They determined genetic structures of clonal mixtures with the aim to form three different classes of heterozygosity. Mating systems were studied both in natural populations and seedling orchards of *Pinus merkusii* (Siregar and Hattemer). Example of the combination of classical breeding and QTL's were presented in the study of Beaulieu *et al.* In their study priority was given to mature wood density due to its high heritability in *Picea glauca* and *P. mariana*. Von Werder and Gregorius brought a modern insight on the application of population genetics to the certification of seed samples and genetic material. Certification of reproductive material could contribute to economic decisions at the forest reproduction.

Based on the discussion to these presentations it was also felt that classical breeding methods and tests need modernization and increasing effectiveness of applied procedures. Incorporation of modern molecular markers and population genetics parameters into the breeding programs is needed, since this should bring a benefit in many ways:

- to assist the early selection,
- to monitor the breeding process,
- to develop effective tools and methods to monitor adaptation and adaptedness.

Concepts and scenarios (Session 8)

Three major issues were addressed in the presentations and during the discussions. The first one concerns “measures and estimation procedures” used for diversity and differenti-

ation assessments (E. Gillet). There was a concern by the audience that different families of diversity statistics are used in genetic inventories making difficult comparative studies across species. In the field of forest genetics, Nei's and Gregorius's parameters are most frequently used. During the presentations and discussion, comparisons were made between the different parameters according to different criteria: estimation methods, statistical tests, inferences in population and evolutionary genetics. Of particular importance was the need of statistical tests to compare estimates within and/or across studies. A method for estimating confidence intervals for delta (the measure of genetic differentiation) was presented. Even if estimators for sampling variance are missing, computer intensive methods (bootstraps) are recommended to be used as alternative ways.

The second major issue addressed the use of simulations in forest tree population genetics (Bialozyt & Scholz). The access to large size memories allows now to use computer intensive methods for testing evolutionary scenarios, for evaluating human impacts on genetic diversity and for upgrading the scale of observations by integrating genetic processes at larger scales. An example of a simulation model was presented where populations are distributed on a grid system and are interconnected by pollen and seed flow. This model can be used for evaluating colonisation dynamics on genetic diversity and differentiation. Simulations models may help to monitor the evolution of genetic diversity and differentiation in non equilibrium situations, since their expectations are only known (from analytical calculations) for equilibrium conditions. Simulations may also help to understand the dynamics of complex multilocus traits (phenotypic traits) submitted under various evolutionary forces. It was recommended that whenever possible, simulation results should be confronted to experimental data. By doing so simulations models may also be used for predictive purposes.

Lastly two presentations considered the applications of genetic investigations in the management of forest genetic resources. It was recommended that a clear hierarchy of objectives in conservation issues and their related activities is recommended before implementing genetic research aiming at designing conservation strategies (S. Herzog). Following system orientated principles, a set of internal and external forces and conditions, respectively, was presented which enable biological resources to withstand irreversible diminishment (H.-R. Gregorius). An attempt was made to define sustainable and unsustainable treatments of biological resources.

Concluding remarks

Lengthening of the growing seasons and other trends clearly indicate climate change and corresponding modifications of the environmental conditions to which forest tree populations are exposed. The genetic response of individuals and populations, respectively, covers a wide range of phenomena such as mutation, modification of gene regulation, genetic selection, loss of genetic variation/variability, inferences on mating processes.

Research questions are evident with respect to those parts of tree species genomes linked to adaptive traits. Knowledge has been accumulated about phenotypic res-

