Teagasc/TCD Symposium on Forest Genetics – Abstracts

The following is a selection of abstracts of presentations at the Teagasc/TCD Symposium on Forest Genetics: Strategies for the Improvement of Forest Tree Species, held on the 9th of March, 1998. Symposium proceedings, to be published by COFORD, will be available in early 1999.

Improving broadleaved species by genetic transformation and utilisation of molecular markers

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Unlike that of annual crops, tree breeding is a slow process. It is, however, of vital importance, given the ever-increasing demand for wood and wood products, and the growing concern regarding the destruction of natural forests. All methods enabling accelerated breeding and all knowledge necessary to optimise the production of tree growth are therefore invaluable. Molecular biology has only recently entered the field of tree breeding, but holds great promise to assist and complement classical tree breeding programmes.

Using DNA fingerprinting methods, DNA markers can be identified which co-segregate with important traits in a pedigree. These DNA markers can be used as diagnostic tools to predict the characteristics of new hybrids long before the traits are displayed, and as such, allow a faster and more intense selection of new hybrids from controlled crosses. The same methods enable the development of detailed genetic maps which enable the unraveling of the complexity of quantitative traits. This research group has identified molecular markers for resistance against *Melampsora larici-populina* (one of the most damaging fungal pathogens of poplar in Europe), for tolerance against *Xanthomonas populi* (which causes bacterial canker), for height growth and for wood specific gravity.

By genetic engineering, it is possible to obtain trees which are ideally suited for a particular purpose without going through the long term breeding programmes. In this field, the group has focused on the alteration of the structure of lignin in poplar by genetic engineering, in order to improve the chemical paper pulping process.

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Range wide variation of genetic diversity and variability of adaptive traits in European oaks: evolutionary impacts identified by molecular markers

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Remarkable range wide trends of variation exist in European oaks for all traits investigated to date. An overall review of current results is presented for sessile oak (*Quercus petraea* (Mattuschka) Liebl.). Geographic trends can be depicted for the level of diversity within a population, and for differentiation between populations. All traits exhibit extremely high levels of within-population diversity. Nuclear markers (isozymes or DNA markers) show only slight allele frequency differences among populations, even among widely separated populations. On the contrary, adaptive traits, particularly bud burst, are highly differentiated among populations. Geographic patterns of variation show opposite trends: nuclear markers follow an east-west trend, while bud burst follows a north-south trend.

Range wide trends of variation are interpreted in terms of evolutionary causes. The comparison of the geographic distribution of quaternary pollen fossil records and chloroplast DNA (CpDNA) polymorphism indicated that Europe has most likely been re-colonised from three different glacial refugia: Iberian peninsula, Italy and the Balkans. Moreover, the patchy spatial structure of CpDNA diversity witnesses founder effects which accompanied post glacial colonisation. Foundation effects were, however, quite rapidly 'erased' by important pollen flow as new colonised areas coalesced into large compact oak forests. These colonisation dynamics are consistent with the contrasted current geographic pattern of diversity between nuclear and chloroplast markers. The potential 'persistence' of an historical effect on adaptive traits was investigated, by comparing genetic distances between populations originating from different glacial refugia with those between populations originating from the same glacial refugium. Results obtained from provenance tests indicate that there is no such persistence, and that current provenance differences for phenotypic traits result most probably from local selection pressures rather than from different historical origins.

Spatial genetic structure and inbreeding in a mixed oak stand based on experimental data and simulations

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Two complementary approaches (based on experimental data and simulations) were used to investigate the effects of natural regeneration on genetic diversity in a mixed oak stand (*Quercus petraea* (Mattuschka) Liebl. and *Q. robur* L.). Two genetic parameters were analysed: the spatial genetic structure in relation to gene flow (by pollen or seed); and the level of inbreeding.

Microsatellite markers were used to investigate the spatial genetic structure at the adult stage and the pollen gene flow with paternity analysis. It was first demonstrated that the

genetic diversity of the adult trees was spatially organised in 'clusters', suggesting restricted gene flow by seed or pollen. Pollen dispersion was characterised by a high amount of long distance dispersion (60% of the pollen coming from outside the 5 ha study area) and by a high variability of the mating success among the pollen donors caused by the distance and direction of pollination.

These results were used as input for a model predicting spatial genetic structure and inbreeding over generations. This enabled testing for the influence of restricted gene flow by pollen and seed on the spatial organisation of the diversity. Various ranges of dispersal parameters for pollen or seed were proposed, from highly restricted to near random. In particular, the influence of asymmetrical gene flow was examined, with a large amount of pollen flow associated with a restricted flow by seed. Results show the establishment of a genetic spatial structure for medium and high gene flow (by pollen or seed). The level of spatial genetic structure is clearly correlated to the level of restriction of gene flow for both pollen and seed. The respective effects of pollen dispersal, seed dispersal and asymmetry will be discussed.

This model also allowed the level of inbreeding in the population to be measured. From an experimental perspective, the estimation of inbreeding is generally indirect (through heterozygote deficiency). This estimation can be biased, but is the only available information on such long lived material where no more than two generations can be followed. An unbiased estimation of the inbreeding was introduced into the simulations, based on the true genealogy of individuals. The underlying assumption was that repeated matings among related individuals could increase inbreeding, especially under restricted gene flow by pollen and/or seed. This has practical implications as inbreeding depression has dramatic consequences in forest tree species for traits such as vigour and fertility. The inbreeding level and spatial genetic structure under various dispersion functions were then measured in parallel. The results indicate that even under strong asymmetry of gene flow, there is only a slight increase in the level of inbreeding.

Breeding strategies for hardwoods: oak, cherry and birch

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Silviculture includes more and more hardwood species all over Europe. This is partly due to ecological reasons and increasingly due to economical factors. Timber prices for indigenous hardwoods are steadily increasing, as the tropical hardwood resources for valuable timber become exhausted and as the appreciation for domestic hardwoods increases. Oak and cherry are traditionally species with valuable timber, and birch has recently reached top prices of more than DM 1,000/m^3 (IR£417/m^3).

Timber quality and growth rate are the key characteristics for a high economic return. Considerable variation exists for these traits between and within populations. Selection combined with appropriate propagation methods offers good opportunities to improve the economic return of hardwood silviculture. Breeding strategies must take into account the biology of the species, time scale and appropriate propagation methods. The approaches

are therefore quite different for the three species under consideration.

Oaks (*Quercus robur* L. and *Q. petraea* (Mattuschka) Liebl.) are long-lived species with late flowering and heavy seeding. While vegetative propagation by cuttings and *in vitro* methods is possible, clonal testing requires considerable time and experience with rejuvenation is still limited. Considerable variation in growth, stem form and other adaptive traits such as flushing, bud set and lammas shoot formation exists within and between populations. Extended populations exist all over Europe. Testing of provenances (stands) and concentration of the harvest of acorns to the most promising sources are the fastest possibilities. Tree selection and seed orchard establishment give an additional gain above the selected and tested stands, however, the seed production is limited. Methods of vegetative propagation can be used to increase the production of plants considerably. In addition, the selection and propagation of superior families and, at an advanced stage of the programme, of clones, are possible. Due to the higher cost per plant from cuttings or *in vitro* propagation, the silvicultural methods have to be adapted.

Cherry *(Prunus avium* L.) is scattered all over Europe in edges of forests, small groups in mixed hardwood forests and sometimes small stands. Cherry has a short life span, flowers early and its seed can be stored for some years. Vegetative propagation by root-sucker is frequent in nature and natural clonal stands exist. Vegetative propagation is comparatively easy and even old trees can be rejuvenated. Clonal testing needs limited time and the natural variability is high for quality, growth and phenology. Provenance testing will be an exception in cherry due to the lack of extended population. Testing of families and the conversion of progeny tests after genetic thinning into seedling seed orchards is an easy and efficient option, but plus tree selection and grafted seed orchards are also working well. The progeny tests offer the possibility to select superior trees from good families and to include those into clonal testing and propagation. Even selected older trees can be rejuvenated by tissue culture and included in clonal programmes.

Birches (*Betula pendula* Roth and *B. pubescens* Ehrh.) are pioneer species with a limited life-span, very early flowering and high intra- and inter-population variation. Vegetative propagation by cuttings and *in vitro* methods is easy and rejuvenation of old trees by tissue culture works well. Species hybridisation with non-indigenous birch species is possible. Some of these hybrids show outstanding growth. The optimal strategies are similar to cherry, however species hybridisation in combination with clonal selection and testing offers a rapid genetic gain. Flower induction by greenhouse seed orchards can speed up seed production in birch considerably.

Some experimental results for the three groups of species are presented, demonstrating the potential for genetic improvement.

Use of microsatellite markers in the management of conifer forest species

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The development of conifer microsatellite (SSR) markers is underway in several laboratories around the world. The Rhinelander Forestry Science Laboratory is developing SSR markers from eastern white pine (*Pinus strobus* L.) and loblolly pine (*P. taeda* L.). The white pine primers, as well as some hard pine primers developed in other laboratories, have been made available for wider distribution.

The white pine markers are being used to compare genetic diversity among native, managed and regenerated stands on the 95,000 ha Menominee Indian Reservation in Wisconsin, USA. Genotype data have been collected for 10 SSR loci in 450 trees, representing nine populations at six sites. Initial analyses indicate that current shelterwood management on the reservation preserves allelic diversity and that heterozygosity is not being lost through excessive inbreeding. Seed samples from two stands having different sawtimber densities are being collected and will be genotyped to determine differences of pollen flows and mating systems between the stands. The information will be used by tribal foresters in forest planning decisions.

As SSR markers are expensive and time-consuming to develop, it would be beneficial to use common primer pairs among related species so that development costs for individual species could be leveraged. SSR primers from *P. strobus*, *P. taeda* and *P. radiata* D. Don have been evaluated for marker amplification in other conifer species. In general, *P. strobus* dinucleotide SSR primers work well in related soft pines, *P. lambertiana* Dougl. and *P. cembra* L., but do not amplify informative loci in hard pine species or in other conifers. Similarly, *P. taeda* and *P. radiata* dinucleotide SSR primers work well in related hard pine species, but not in soft pines or other conifers.

In addition to nuclear DNA SSR markers, SSR markers for pine chloroplast DNA (cpSSRs) are also available. In collaboration with G. Vendramin of CNR, Italy, variation in paternally inherited cpSSRs was used to study population genetic structure in red pine (*P. resinosa* Ait.), a species characterised by morphological uniformity, no allozyme variation and limited RAPD variation. From nine cpSSR loci, a total of 23 chloroplast haplotypes and 25 cpSSR alleles were found among 159 individuals surveyed in seven widely separated populations. All populations could be distinguished from each other by their haplotype compositions. Frequency distributions of pairwise SSR differences among individuals within different populations indicated recovery from one or more population bottlenecks, and support a metapopulation concept for the species. In collaboration with B. Epperson of Michigan State University, more extensive sampling of red pine populations is proceeding, and cpSSR and nSSR data will be analysed to determine historical routes of gene dispersal and to quantify the range-wide geographic pattern of genetic diversity of natural red pine populations. This information will be used to preserve and utilise what little genetic diversity remains in red pine.

Chloroplast microsatellite approach for the study of diversity in conifers

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Chloroplast microsatellites (cp-SSRs) are highly polymorphic markers which can be used efficiently to study the history of populations in the most recent post-glacial period, for the analysis of the distribution of haplotypic diversity within and among populations, for the detection of natural hybridisation among different species, and for paternity analysis in conifers. The high degree of sequence conservation in the chloroplast genomes of conifers gives these markers the property of 'universality', whereby primers have been designed which allow for their cross-amplification among species and genera. By this means, the cost of developing markers has been reduced substantially. Here, results obtained using chloroplast microsatellite markers in *Pinus pinaster* Ait., *P. halepensis* Mill., *P. brutia* Ten., *P. resinosa* Ait., *Picea abies* (L.) Karst. and *Abies alba* Mill. are presented and discussed.

Occurrence and detection of triploids by microsatellite analysis

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Triploidy occurs frequently in nature but most of the time it does not result in viable organisms. Viable and fertile triploids do sometimes occur, and these may display advantageous characters compared to diploids.

In trees, polyploids including triploids have been recognised for some time. Polyploids are rare in the gymnosperms and abundant in the angiosperms, where they represent a third of all the tree species. The development of cytological techniques allowed the detection of polyploidy in trees as early as 1927, and ploidy assessment in trees was extensively investigated during the period 1935-60. Forest scientists in that period expected to increase productivity by creating triploid or polyploid clonal lines which could be valuable in terms of productivity. The development of biochemical tools such as isoenzyme analysis in the 1960s and '70s also showed the effects of ploidy changes.

More recently, molecular markers such as AFLPs, RFLPs, RAPDs and specific gene markers have been applied to forest trees. These have also shown evidence for changes in ploidy level as a possible explanation for certain molecular patterns. Molecular methods can now be used to detect or confirm changes in ploidy, when used in conjunction with

cytological and other morphological traits such as stomata length. Reported here for the first time is the confirmation of triploidy in a confirmed triploid oak, as well as the occurrence of polyploidy (triploidy/aneuploidy) among elite Irish oaks. This research was carried out using microsatellite DNA profiling, which is the most recent method for analysing genetic polymorphisms at the DNA level.

This paper reviews the historical and recent literature on polyploidy in trees, and its use in programmes for tree improvement.