EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

Conservation of Forest Genetic Resources in Europe

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J. Turok, C. Palmberg-Lerche, T. Skrøppa *and*
A.S. Ouédraogo, *compilers*

International Plant Genetic Resources Institute
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IPGRI is an institute of the Consultative Group on International Agricultural Research (CGIAR)

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Proceedings of the European Forest Genetic Resources Workshop
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The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI's headquarters is based in Rome, Italy, with offices in another 14 countries worldwide. It operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme, and (3) the International Network for the Improvement of Banana and Plantain (INIBAP). The international status of IPGRI is conferred under an Establishment Agreement which, by January 1998, had been signed and ratified by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mauritania, Morocco, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

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The European Forest Genetic Resources Programme (EUFORGEN) is a collaborative programme among European countries aimed at ensuring the effective conservation and the sustainable utilization of forest genetic resources in Europe. It was established to implement Resolution 2 of the Strasbourg Ministerial Conference on the Protection of Forests in Europe. EUFORGEN is financed by participating countries and is coordinated by IPGRI, in collaboration with the Forestry Department of FAO. It facilitates the dissemination of information and various collaborative initiatives. The Programme operates through networks in which forest geneticists and other forestry specialists work together to analyze needs, exchange experiences and develop conservation objectives and methods for selected species. The networks also contribute to the development of appropriate conservation strategies for the ecosystems to which these species belong. Network members and other scientists and forest managers from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme. EUFORGEN is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries.

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Introduction

Christel Palmberg-Lerche
Chief, Forest Resources Development Service, Forestry Department, FAO, 00100 Rome, Italy

Jozef Turok
EUFORGEN Coordinator, IPGRI, 00145 Rome, Italy

The geographic region of Europe includes ecologically diverse, temperate, boreal and Mediterranean types of ecosystems. However, in spite of environmental differences, many of the tree species found in Europe have a wide distribution range across the continent and are shared among many countries. Furthermore, European foresters share similar traditions in silviculture and forest management. This provides a common basis for incorporating present-day concerns into forest management practices.

In recent times, forests in Europe have not experienced drastic loss of tree species as a result of human action. However, native forest tree populations have been subject to human impact and intensive utilization for centuries, which have had both positive and negative effects on their genetic resources. The introduction of species and provenances from other countries and regions for the establishment of forest plantations has also had an impact. A recent concern in the region has been forest decline attributable to changes in the environment and, above all, to industrial atmospheric pollution. Privatization of forests and other developments in countries with economies in transition have also created new challenges.

The European Forest Genetic Resources Workshop, organized by IPGRI in technical collaboration with FAO in Sopron, Hungary in November 1995, provided an opportunity to bring together specialists and practitioners involved in forest genetic resources activities in different parts of Europe. The one-day Workshop was held immediately following the first Steering Committee meeting of the European Forest Genetic Resources Programme (EUFORGEN). Discussions at the Workshop were targeted to enable European countries concerned with forest genetic resources to provide an input to the International Technical Conference on Plant Genetic Resources, held in Leipzig, Germany in June 1996.

Delegates from 28 countries attended the Workshop, representing Austria, Belarus, Belgium, Canada, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Malta, Moldova, the Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Turkey and the Ukraine.

The Workshop was organized around three technical sessions: (i) strategies for the conservation and use of forest genetic resources in Europe, (ii) practical experiences and approaches used in the different ecological regions of Europe, and (iii) conclusions and recommendations.

The official report of the present Workshop was published immediately following the meeting.[1] As the eight technical papers presented at the Workshop provide a good overview of information on forest genetic resources strategies and are a particularly useful source of information on experiences in this field in Europe, it has been decided to publish these papers to complement the brief official report, and to make this valuable information more widely available.

The first three papers outline general strategies for the conservation and sustainable use of forest genetic resources in Europe. The following five papers complement these by presenting a review of specific issues related to north European boreal forests (two papers), forests in central Europe, forests in the Mediterranean and forests in the former Soviet Union.

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[1] Part III *in* IPGRI/FAO. 1996. International Technical Conference on Plant Genetic Resources: Preparatory process for Europe. IPGRI/FAO, Rome, Italy.

In formulating recommendations to the Leipzig Conference (see Appendix), the Workshop considered the outcome of the European Regional Meeting on Plant Genetic Resources held in Nitra, Slovakia in September 1995, as well as those of two other workshops on forest genetic resources (Berkeley, California, June 1995; and Toronto, Canada, June 1995). It was emphasized that the recommendations made by the Workshop should be considered as being complementary to those passed at these meetings.[2]

Based on the technical presentations and subsequent discussions, the Workshop participants stressed that strategies and methodologies applicable to the conservation and sustainable use of forest genetic resources differed decisively from those used for agricultural crops. Forest trees are largely undomesticated and contain substantial levels of genetic variation which should be conserved, managed and utilized to meet the needs of present and future generations. It was further noted that forest ecosystems provide a range of products and environmental benefits, such as timber and other wood and non-wood goods and commodities, genetic materials of both woody and non-woody species, protection of soils and water, and use for recreation. As the management of forest genetic resources needs to take into consideration the maintenance and enhancement of such products and benefits, strategies aimed at conserving forest genetic resources are clearly much broader in scope than those for agricultural crops.

It was emphasized that the conservation of forest genetic resources required a clear statement of objectives and dynamic management. Neither natural ecosystems nor breeding programmes are static and genetic conservation should not aim to freeze a given arbitrary state of dynamically evolving, living systems, but to ensure that the evolutionary potential of species and ecosystems is maintained and enhanced.

Efforts to conserve and enhance forest genetic resources for present-day and future use should include the management of protected areas, productive forests and breeding populations. This 'tripod' of action offers the only lasting solution to the challenge of conservation. The key to success is vested in the development of programmes which harmonize conservation and sustainable use of forest genetic resources within a mosaic of land-use options, and which include a strong element of active management. Sustainability of action over time should, furthermore, be based on genuine efforts to meet the needs and aspirations of all interested parties, and requires the close and continuing collaboration, dialogue and involvement of all stakeholders in the planning and execution of related programmes.

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[2] The International Technical Conference on Plant Genetic Resources for Food and Agriculture held in Leipzig, 17-23 June 1996, adopted a Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture which stated in its introduction: *"Forestry will not be included in the Global Plan of Action ... on the understanding that this matter could be considered in future, in the light of the work of the Intergovernmental Panel on Forests (IPF) established by the Commission on Sustainable Development on this issue."* The IPF did not address the issue of forest genetic resources. However, following discussions at the Thirteenth Session of FAO's Committee on Forestry (March 1997), FAO was requested to assist countries in the organization of a series of country-driven and action-oriented regional and sub-regional forest genetic resources workshops, to be carried out in collaboration with IPGRI, ICRAF, CIFOR and other international partners, with a view to develop a coherent global framework for action in the conservation, management, sustainable use and enhancement of forest genetic resources. An FAO/IPGRI/ICRAF workshop was organized in collaboration with the DANIDA Forest Seed Centre for dry-zone Sub-Saharan African countries in September 1998.

Presentations

Forest genetics and conservation in Europe

Gene Namkoong
Department of Forest Sciences, University of British Columbia, Vancouver, B.C., V6T 1Z4, Canada

Introduction

There are two commonly held views of gene conservation that guide conservation programmes. The agricultural view assumes a high level of agricultural technology, the use of rapid breeding techniques for specific traits, stored genes and seed collection as the dominant form of gene conservation. The second is that natural regeneration has endowed populations with an optimum and stable state of the resource and that preservation of that natural endowment is the best form of conservation. I will argue that:

- while popular, neither point of view is feasible for forest gene conservation as they are both based on the assumption that the genetic resource is static and that conservation can only delay the loss of genes and species;
- the resource is in a dynamic state and an active programme of conservation and use is necessary for an effective conservation;
- the genetic resource has changed in historically constrained ways and rarely achieves an optimum 'adaptedness', but the present state of the resource is the initial condition on which selection towards adaptation and the other forces of evolution operate.

Therefore, instead of considering the present state as an objective to conserve, a feasible gene conservation strategy should consider what differences exist between the present state of the resource and the condition that it is reasonable for us to seek. For forest geneticists the strategy would focus on developing genetic management plans to bridge the gap between what exists and what is sought. This would seem to require that we collate what is known or knowable about the present condition, define the condition that we seek, and consider the means we have available for going from one to the other condition.

If the resource were stable and static, it could be assumed that an optimum adaptation already exists and need only be perpetuated. We would not need to know much about the resource or its evolution, just how to perpetuate it, or how to identify the genes that can close the gap between the present and the desired type of organism. There may be only a small gap between the present and the desired conditions but a gap that we would know could be efficiently closed with present technologies. This is close to what the practice for agricultural crop species and the problem of conservation was up until 1972, when it was found that the genetic resource available to many crops was deteriorating with the loss of genetic diversity in breeders' populations. The task for applied plant geneticists was then largely defined in terms of supporting or restoring the presumed original condition, or at least of saving all potentially useful genes. Relatively little attention was given to the evolution of the breeding system itself or to the breeding objectives.

However, the world of forest geneticists is neither so simple nor as clearly defined. While the same kinds of deterioration in the endowed resource may exist as for agricultural crops, the goals of genetic management are far more diverse, the quality of environmental control over both time and space is far less, and breeding cycles are much longer. The products and values generated by forests involve the standing vegetation and the various stems, fruits and other harvested parts. The ecological variety of conditions within single forests and within forested landscapes are in areas considered unsuitable for agriculture and, therefore, only include many sources of variation and wide ranges of those variables. The time scales over which conditions change vary from days to decades but are dominated by the life cycle of trees. In addition, even the world's ancient forests are known to undergo continual change (Willis 1993), and few, if any, forests can be considered static in terms of their genetic or species composition. In general, the world's forests must have been massively altered from any possible pristine state, and hence no naturally stable or static system can be assumed to exist. Therefore, forest genetic resources do not start with the simple systems assumed for the above two models, they do not have the same end goals, and they must be managed by different means. If forest geneticists are to achieve feasible goals a unique system of gene conservation must be developed.

The state of the resource in Europe

One view of European forests is that they are an exception because of impoverishment caused by glaciers. With relatively few species that are known to have simple outcrossing systems, the structure of variation could be simple and could have been stabilized by heterozygote superiority or such wide migration that a stable average optimum holds sway. Therefore, the genetic system may be viewed as being very simple and would imply a simple sampling problem for conservation.

Selection may also be viewed as being uniform or simple, so that neither selection nor migration is a problem for conservation. In fact, one of the views of selection in European forests is that there is only one factor that dominates adaptation and that is cold temperature. This surely affects whole species similarly and must have consistently done so for several thousand years. The effects of cold on various organs and tissues may require different physiological responses and phenologies, but with independent gene effects and with imperfect genetic correlations among traits, multiple trait fitness optimalities can be expected to have evolved. Thus, with high migration rates that would homogenize allele frequencies and with selection factors simply condensable to one variable, alleles may be distributed uniformly or at most, along only one climatic axis. Then, conservation would only require sampling along a single gradient.

It is, therefore, tempting to simplify conservation efforts in Europe to a programme of storing some finite number of seed samples to save useful alleles. An average fitness would have been maximized, and if any local differentiation in selection pressures exist, the populations could not differentiate much anyway. It would then be safe to assume that no important linkage groups (co-adapted gene complexes) exist, that all alleles are widely spread and that samples taken at the ends of the extremes of the climatic variable would save all alleles. The only problem is sample size needed to satisfy minimum probabilities of loss (Gregorius 1988; Namkoong 1991), and these sizes are well known and come to only a few thousand trees or seeds. If there are doubts that seed collections may be subject to further genetic erosion, then it is only required to increase sample sizes by some relatively small factor.

In order to use this endowed variation, the agricultural paradigm is appealing. Wide sampling and storage of the variation would once and for all save nearly all alleles, and since breeding could then use direct gene identification or markers, the development of new varieties is merely a problem of assembling the desired characters. The reductionist paradigm is then nearly complete.

It is possible that a conservation programme can be relatively simply constructed with little strategizing needed, if indeed that resource evolved simply, stably and optimally, and if the use of the resource only required clever assembly. Before proceeding with such a programme, however, it might be useful to examine these assumptions about evolution and utilization, and to consider if any factors may change programme recommendations.

Evolutionary history

Unlike tropical forests, natural selection in European forests has been strongly affected by soil and climatic variables with relatively less intense competition from other plants. However, this implies that the forms of selection in these forests have been neither simple nor consistent. Over the past 10 000 years since the hypsithermal, there have been episodes of warming and cooling, wetness and drought, and advances and retreats of ranges in northerly, southerly, easterly and westerly directions; the mechanics of advance are not the same as those of retreat. Most species have occupied their present ranges in Europe for fewer than a hundred generations and then often only intermittently. Multiple climate shifts can be expected to have caused large range changes and hence selection is likely to have been strong. Pockets of vegetation that lie at the edges of forest and tundra on the one hand, and forest and field on the other, may either be advancing frontiers of long distance migrants, or retreating relict populations.

Migration

For species that have invaded the regions of central Europe, there must have been an historic constraint on the availability of plant families and genera from which migrants and genetic sources for speciation events could be drawn from border populations or internal refugia. However, once established as invaders, they form communities that are themselves resistant to invasion, and within species variations may develop in response to mutation, new migrants from refugia and new selection pressures. There are no forms of inbreeding that are especially frequent, nor is there any lack of inbreeding depression and, hence, no reason to believe that special co-adapted gene complexes exist in these species. These species do not seem to be differently structured from other species in temperate-zone forests.

The view that we can derive of the evolution of the European forest, therefore, seems to be one of a genetic and reproductive system initially constrained to remnant or highly migratory pioneer species, which are now relatively undifferentiated, although highly variable genetically. Species in the complex of advancing and retreating fronts would be under high selection pressure for climatic adaptation in latitudinal, longitudinal and elevational gradients, and to soil conditions, most of which would have been affected by glaciation events. Thus, while these species have the genetic variation that would enable rapid evolutionary response to selection for special adaptations, such responses to selection are constrained by high genetic migration rates. Therefore, the patterns of geographic variation so often observed in growth and reproduction traits must be the result of very strong selection pressures that can overcome the homogenizing effects of wide migration.

Selection

Different limiting factors must have dominated evolution of local populations, for example nutrient stress, soil depth and frost-free periods. Migration routes of pioneer species would have been affected by prior vegetation and the conditioning effects they had on the soil and competitive environment; hence routes blocked or opened by multiple historic factors. The speed of migration and the particular paths may thus have been variously subjected to environmental constraints provided by more pioneer vegetation, or by the existence of environments and soil conditioned by retreating competitors. Thus, selection factors of the environment could be vastly different for the same species in the same kind of forest-tundra border, but responding to different limiting factors and in different parts of the cycle of advance and retreat. I would suspect that there are concomitant differences in the annual allocation of resources within trees between organs and tissues, and that age-dependent processes also differ within these species. Thus, the relative selective effects of latitude, longitude and altitude on the evolution of divergent population behaviour are expected to differ among traits. Therefore, even if a single species can inhabit a wide geographic range, as do most trees native to Europe, strongly divergent and complex selection pressures must be expected to exist whether that pattern is exhibited in gene frequency divergence or not.

If the tundra edges of the forest were unstable, then also the wetland-forest transition would have been no more stable, and the subtropical-temperate forest border must have been even less stable. The vegetation of those regions was also advancing and retreating over large distances and responding to temperature, soil, fire and other biotic elements such as insect devastation which seem to have been widespread. For all of these reasons, the likelihood that forests were ever in a stable condition seems very small, and cyclic behaviour, as well as aperiodic fluctuations in the physical and biotic environment, must have been common. Therefore, unstable selection forces must also have a destabilizing effect on the genetic system of any forest tree species in Europe.

Under such conditions, it can also be expected that geographically related selective patterns of gene frequency changes would not be the same for all loci. One consequence of this for gene conservation is that if we wish to sample different alleles that have been subject to divergent selection, we would have to use several environmental surrogates and sample genes from extremes in more than one environmental variable. If the environmental variables were not identically distributed, then divergent sampling schemes would be more efficient than a common scheme. It should also not be surprising that isoenzyme variation patterns are often found to differ among themselves and to differ from those of RFLPs, from RAPDs, and from physiological and morphological traits that are closer to direct selection effects. The strong homogenizing effect of high migration rates is only overcome by strong selection or by temporary drift effects in small populations. Since different selective pressures must have been large in the recent past, we have little reason to believe that divergent selection pressures will be any more moderate in the near future.

With strong and highly variable selection forces, it is conceivable that species would have responded with a highly homeostatic response mechanism affording all of its individuals with similar levels of plasticity in component traits. If this kind of response uniformity evolved, then all trees would respond similarly to site variations. In fact, it is a research challenge to demonstrate that genotypes of European species have more constant norms of reaction and less genotype–environment 'interaction' than other temperate or boreal zone species. Instead, it seems that there is a substantial degree of provenance variation in response to planting environments, and clines exist in growth, reproductive behaviour and edaphic responses, demonstrating differentiation in many traits. Therefore, there is at present little indication that the tactic of developing strong individual level homeostasis has been evolved. In fact, we are familiar with strong provenance variation in many traits and in the general existence of high provenance by environment interactions. Yet, isoenzyme variations show relatively little population differentiation while rather high levels of genetic variation persist in most populations and species for both physiological traits as well as isoenzyme loci.

The pertinent evolutionary question is how this genetic system evolved and whether there are alternative evolutionary paths that could have been taken. It would seem that most European tree species are forced by their mating system to compromise in their response to site variations by not adapting very specifically nor very quickly. Instead, they endure a constant tension between local adaptation and the swamping effects of gene migration, which results in a strong genetic inertia and maintains a large reservoir of variation with a large lag time in long-term response.

Evolutionary future

The long-term future will involve much the same kinds of selective forces in climatic changes and their consequent complexes of different environmental limiting factors including soil, insect, fungal and other biotic factors as well as temperature and moisture regimes. We are now in a period of gradual cooling within an interglacial, but also within a cycle of smaller variations in warming and cooling. Of great concern at present is also the strong anthropogenic effect on land occupancy, atmospheric composition and stratospheric ozone. Large variations have been experienced in Europe in the past, and larger changes are predicted for the future. There are also more rapid cycles of human use and impact on forests which will change the conditions and the goals of gene conservation. We can expect that there will be factors that could result in more homogeneous forest populations and those that will further diversify future forests. One of the greater certainties that we have for future conditions is that there is considerable uncertainty concerning even the conditions that may require more homogeneity. Therefore, the conservation problem is to ensure the capacity of forests to adapt to highly variable conditions at any one time and to changes in the array of conditions that may appear over time. Hence, the capacity of the system to evolve must be conserved together with the capacity to adapt to local variations (Eriksson *et al.* 1993).

The traditional gene conservation programme is one of saving all presently existing alleles either in seed banks or in 'natural' forests. If the distribution of alleles is uniform, then large sample sizes are needed to save rare alleles but no geographic distribution of samples would be useful. Large collections are better than small ones and we can judge the quality of such a conservation programme by the number of accessions it contains. The sample design would then be irrelevant to the probabilities of saving alleles. If, however, the distribution is random and, therefore, clumped but in unknown patterns, then grid sampling would increase the probability of hitting a clump of high frequency and of saving such alleles. If the distribution is clumped because of historic or selection factors, then clumped sampling weighted by probability of hitting high frequency clumps is feasible.

However new alleles are created, some go to extinction and others vary in frequency. Selection, mutation and migration will influence future evolution, just as in the past. Mutation rates are variable among loci and can be increased by various exogenous treatments as well as by rate-enhancing techniques. If new mutations are to be saved, then increasing their frequency is desirable and can be achieved by selecting and detecting them at the gene level or at the physiological trait level. In addition, the probability of losing undetected alleles increases in small populations, but if detected and selected for, the probability of their being saved in at least one of many populations can be higher than in a single large population of equal total size without selection. Thus, future evolution can operate on higher numbers of new alleles than historically existed.

Migration can also be managed within species to increase divergence among populations, and since future evolution will induce species migrations, it is possible to manage the physical movement of diverged populations on a far greater scale than by unaided evolution. Therefore, the previous cooperative provenance tests between Europe and North America may be expanded to include Asian and South American species. In addition to such 'east-west' migrations, some 'north-south' migrations of more temperate species and populations can be included in a gene conservation programme.

If migration at the species and population levels can be manipulated, and the strength and diversity of selection controlled, then genetic management can have a strong effect on future evolution. New solutions can be developed to solve the evolutionary problem of how species can fine-tune local adaptations and still maintain a genetic capacity to respond to long-term changes in the selective environment. Previously, evolution has always produced regression to the mean and constrained the genetic variance so that local adaptability was sacrificed for a species-wide, general fitness. By simultaneously managing migration and selection, we can increase the total genetic variance, and the choice of which population is deployed in local regeneration is no longer dependent upon locally available parents only. We can use multiple population sources of wider divergence than previously available in single or mixed regeneration and can choose from, and plant, genetically more diverse populations than could exist without such a genetic structure. A variety of techniques exist including intensive-breeding seed orchard programmes to seed stand selection to manage the genetic structure, and with the aid of molecular fingerprinting, rapid and inexpensive techniques are available.

Since the effects of large population size on the probability of saving alleles is logarithmic in form, while the costs of sampling and maintenance are more nearly linear, trade-offs will exist between-population size and numbers of populations. Efficient sampling and genetic management within limits can trade individual population size for multiplicity of populations and species. After the first few tens or hundreds of genotypes, the benefits of random accumulation of samples rapidly diminish relative to their cost. The advantages of sampling, maintaining and managing multiple populations seem obvious. In multiple populations, selection can effectively diversify populations. Whether selection is for commercial objectives or to generate different combinations of traits designed for adaptability to a broad variety of possible future conditions, the same forces of selection, migration and mutation that evolved forest tree species in the past can be used to guide their future evolution. Thus, conserving the adaptability of the genetic system requires the conservation of the evolutionary capacity of forests, which requires a substantially different strategy than one of saving 'all the parts' or alleles of the present condition. The problem for gene conservation is to design a system that can do at least as well as doing nothing.

If forest genetic management is practised in the future, then the structure of genetic variation can be managed for future adaptability. One objective may be to increase homeostasis at the level of the individual tree so that at least as wide a range of conditions as is presently accommodated can be accommodated also in the future. Another is to allow little or no reduction in individual homeostasis but to increase the variation among populations, and to increase the number and range of correlated responses that may exist among populations. The relative advantages of each, or the best levels of joint increase, is yet to be studied. However, a programme in Sweden and one in Canada are beginning to develop options for managing the genetic variances and covariances between and within populations.

This mode of conservation does not reduce variation to guesses about the exact nature of future conditions and, therefore, does not increase risks of extinction associated with misdirected selection caused by errors of prediction. It is instead pro-active in generating genetic variances more efficiently than by random accessions based on present allelic distributions.

A conservation proposal

In contrast to agricultural gene conservation, in forestry we do not have the hierarchical arrangement of breeding populations that rapidly reduces the genetic variance in populations to adaptability to a highly controlled set of environments. Neither do we have annual or shorter breeding cycles nor the vast difference between cultivated and wild populations. Instead, we have a much broader array of public interest to protect in the crops produced and in the benefits of heterogeneous conditions. Therefore, while agricultural systems might offer one model of how some of the requirements of a genetic conservation system might be fulfilled, forest trees demand a more comprehensive system. Since forest genetics is concerned with many species at the beginning stages of management and domestication, and a broad range of values and products, a forest gene-conservation programme must be far more inclusive than agricultural programmes.

The agricultural system reviewed recently by the US National Academy of Sciences includes a series of collections of important crop species and their near and wild relatives. These are used to infuse breeders' populations with alleles and variations that are used in crosses and backcrosses to isolate specific enhancements to well-adapted populations. More rarely, whole new varieties are added to the genomic repertoire, and more rarely still, new species are developed to the point that they can substitute or add to the agricultural system.

Various breeding schemes use hierarchies of populations to maintain variation in populations accessible to breeders.

With few exceptions, gene conservation in the agricultural system has not emphasized the active development of the base breeders' populations with independent genomes, because of the large separation between the crops and their wild relatives. In forest trees, the differences between wild and cultivated varieties is still small and an alternative form of conservation has been to simply rely on nature conservation to provide for any future needs. For some forest tree species, one or the other of these two choices may be sufficient. Hierarchies as well as multiple population 'factorial' sets of populations are being developed for those few dozen species which are at present of sufficient commercial interest to support breeding programmes. A few dozen more species are included in species and provenance trials, but further efforts to include genetic surveys of more than these few dozen species are not organized. In addition, there is a broad network of forest reserves, parks and national forests that contain most of the widespread species and even if genetic surveys are lacking, these may contain most of the genetic variation of those species that presently exist. What seems to be missing is a system-wide organized view of the structure of genetic variation, the targeting of resources which may be at risk, and the costs and benefits of conserving them.

If we presently lack such a system, can we continue to conserve genetic resources without a global system? In the future, it may prove that for most species it will not be necessary to do more than conserve a few samples, that present programmes are adequate to ensure their continued existence, and that no values are lost, or opportunities foregone, by merely maintaining a few such collections or *in situ* conservation sites. For those species that justify intensive development, it may also prove fortuitous to have captured the useful variations, and the development of future variances and covariances will be managed using present resources. However, until this is known, it would seem desirable to ensure that a global system exists. Minimally, it would seem feasible to investigate those species about which it is reasonable to fear that the resource is not being conserved or which may fall into a high-risk category, and for which proactive conservation would be effective.

To organize an inclusive European gene-conservation effort, species might be divided into categories of present, potential and of unjudged value for either economic or ecological goals. For those species of known present value, more detailed knowledge of their genetic structure is useful for their management and hence, detailed information should be available, or programmes instituted, to obtain such data for all types of genes and traits. For these species, conservation of wild relatives and extreme variants can be targeted because they may provide support for future intensive programmes. The FAO Panel of Experts on Forest Gene Resources lists of species, and the previously established breeding and provenance programmes, form a basis for these efforts.

For species of little present, but of potentially high future value, those species may be targeted where the risk of losing potentially valuable alleles and variation may be high. In such cases survey and sampling is desirable, especially for those whose distribution range or demography puts them at potential risk. Species or populations at the edges of their ranges of adaptability would form good targets for special interest even though they may be of little present value. Such populations may never be intensively managed, but they may be targeted for maintenance in some reserve status in case their future value increases and justifies higher management intensity.

For those species or populations of unknown value, genetic surveys may be useful if only to confirm that they will be able to exist in future environments in a tertiary category of interest. Species and populations that may be small in size and range, or that fall into a category of endangerment that can be more easily prevented than later repaired, would be targets for initial sampling if not collection. Especially for species and populations where in view of limited conservation funding only relatively low maintenance can be justified, ecosystem conservation programmes and agencies, rather than genetic conservation programmes, will hopefully be the focus of effort. For these species, which represent the vast majority of woody species, population size and management are likely to be the primary means of ensuring their survival and evolution, while for the first two categories, genetic considerations and agencies are likely to dominate conservation planning.

A conservation strategy for European forests could, therefore, be built around the FAO Panel of Experts lists, plus the breeding and provenance research that have already been organized, and the ecosystem conservation programmes of governmental and non-governmental agencies. What seems to be lacking is a comprehensive mechanism to ensure that all species are being adequately managed and that all of the various questions about past and future evolution that constrain and define gene conservation goals are considered.

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Setting priorities in conservation of genetic diversity — with special reference to widely distributed conifer species

Csaba Mátyás
Department of Environmental Sciences, University of Sopron, 9401 Sopron, Hungary

Introduction

Any scientifically founded conservation strategy has to be based on clearly defined objectives. Although motivations for conservation of forest genetic resources are manifold, the pressing need for action at present results from forecasts as to the effects of global changes on the environment in Europe.

There is a broad spectrum of stress factors menacing forests both at present, and in particular, in the near future. These will influence conservation strategies as the principle of traditional conservation, i.e. avoiding any type of disturbance, diminishes in view of forecast uncertainties and changes (Ledig and Kitzmiller 1992; Eriksson *et al.* 1993). The expected climate instability and sedimentation of pollutants are important indirect factors influencing both the species composition and gene frequencies within species, even in totally 'undisturbed' communities. Certain activities, such as forestry and other measures affecting site conditions and gene frequencies directly (e.g. water regulation) may be adjusted to conservation needs. However, a considerable amount of stress resulting from changing site conditions will have to be accepted and reckoned with, as indirect effects of human activities cannot be stopped at the boundaries of designated reserves. The speed and nature of these changes is uncertain but they may render an idealistic concept of gene conservation useless. Therefore, the selection of priorities should be an important task for the formulation of realistic strategies.

It is probably undisputed that the central task of gene conservation is, beyond any economic thinking, the maintenance of the evolutionary adaptive potential of species, communities and ecosystems. This is no different in forestry; thus the evolutionary viewpoint must receive proper attention. An additional argument in the case of trees is their long life cycle, which makes policy changes on a year-to-year basis impossible, and which renders decisions once made, practically final and irreparable.

Widely distributed temperate conifers have been selected for the discussion of possible needs and tasks for two reasons: a great amount of data and information exists on the genetic structure of these species; and the task of gene conservation seem less urgent in their case, compared to tree species with limited and fragmented distribution.

Status quo preservation: principles and problems

Conservation of indigenous genetic resources (and forest communities) through *in situ* methods, i.e. *status quo* preservation is based on the concept that under natural conditions local optima of allelic frequencies evolve which best fit the given environments. It is also presumed that indigenous local populations (and forest communities) resist environmental stress and diseases better than others do. This concept implicitly includes the view that the original genetic diversity as a whole is the resource that has to be preserved. Therefore, there is no justification in trying to attach values to certain traits, as the fitness value of traits is very difficult to assess and even obviously neutral traits or genes may gain in importance in a different selection situation. As a consequence, the genetic constitution of naturally developed indigenous populations should be kept unchanged and as concise as possible.

The aims of conservation appear in this case relatively simple: based on genetic inventory conservation strategies and methods have to be developed in a way to ensure the survival of locally adapted populations and to avoid the loss of rare alleles. If the necessary information and funding are available, it is difficult to argue with this concept. Even in this ideal case, however, some doubts may arise, such as:

- In Europe, criteria for 'undisturbed natural conditions' are seldom met even in the most remote areas because human activities in forests were often intense in the distant past (e.g. slash-and-burn agriculture even at high elevations during warmer climatic periods, extensive forest clearing around mines; charcoal and potash production). In most cases, the forest tree populations existing at present are not purely the product of natural selection processes.
- Even in areas where direct human interference has been totally banned, certain indirect anthropogenic effects will continue to exert their influence on the genetic constitution of the populations, such as damage caused by uncontrolled game stocks and atmospheric deposit of pollutants. Therefore, an unbiased action of natural selection forces cannot be anticipated.
- This approach offers no clues on how to handle tree populations of human origin or in general, exotic species, as both are considered valueless.

Species and gene migration and the need for human interference

It could be argued that irrespective of certain not really 'natural' selective effects, if large enough, *in situ* conserved forest communities will keep pace with changes and evolve to counterbalance these changing scenarios by adjusting gene frequencies within populations and species composition within communities. In this respect migration seems to offer options. On the most threatened southern or arid limits of vegetation zones it will be species change through migration that has to stabilize the forest communities. For this, a migration speed matching the pace of expected changes is needed. For central European conditions it could be shown that according to the scenario of a temperature increase of from 1-1.2°C in the year 2030, isotherms would wander in northerly direction with an approximate speed of 6-7 km/year (Mátyás 1996). This has to be compared to the migration speed of species. It is known from paleobotanical studies that tree populations migrate, depending on migratory abilities, at rates of from 0.1-0.4 km/year. It is obvious that the difference is more than one of magnitude. This means that even where migration routes are available, which is seldom the case in the intensely fragmented landscape of Europe, geneflow through natural migration of species populations is practically out of the question.

There is, on the other hand, no information on the migration effectiveness of alleles within a contiguous distribution area. Judging from gene frequency patterns, this speed might be considerably higher than the migration of the populations. It may be assumed that within contiguous ranges the speed of gene frequency adjustment through migration of pollen could be sufficient to match the pace of changes. Preconditions are: an unfragmented distribution of the species, the overall natural state of populations and the general application of natural regeneration. Neither of these can be presumed in Europe, except possibly in certain parts of the boreal zone. This implies that the need for artificial conservation measures is most pressing in the southern peripheries of the respective vegetation zones, but if the above-mentioned preconditions are not met, then practically over the whole present range. If these considerations are correct, human help will be also needed to extend the ranges of species at the northern peripheries of zones. This will be the case especially in the northern boreal zone where possible temperature increases may be considerably higher than at medium latitudes.

An aspect usually forgotten in species migration modelling is soil quality. Especially in the north, soil development will certainly not follow the pace of expected warming, so sites will not automatically improve in parallel to temperature increases.

Suboptimal adaptation: an issue to be considered for conservation strategies

There is growing genetic evidence that the result of natural selection is most probably far from optimal, i.e. adaptation to local conditions is imperfect. It seems that it lies in the nature of the adjustment process that the directional gene frequency changes within the population, by definition adaptation itself, has clear biological constraints. The systemic-genetic reasons hindering the achievement of maximum fitness are numerous (Loeschcke 1987). Suboptimal adaptation also results from random effects caused by the migratory past of the species as well as human interference (selective cutting, transfer of reproductive material). The effect of evolutionary and adaptive constraints contribute to the genetic load of the population, termed as 'adaptation lag' (Mátyás 1990).

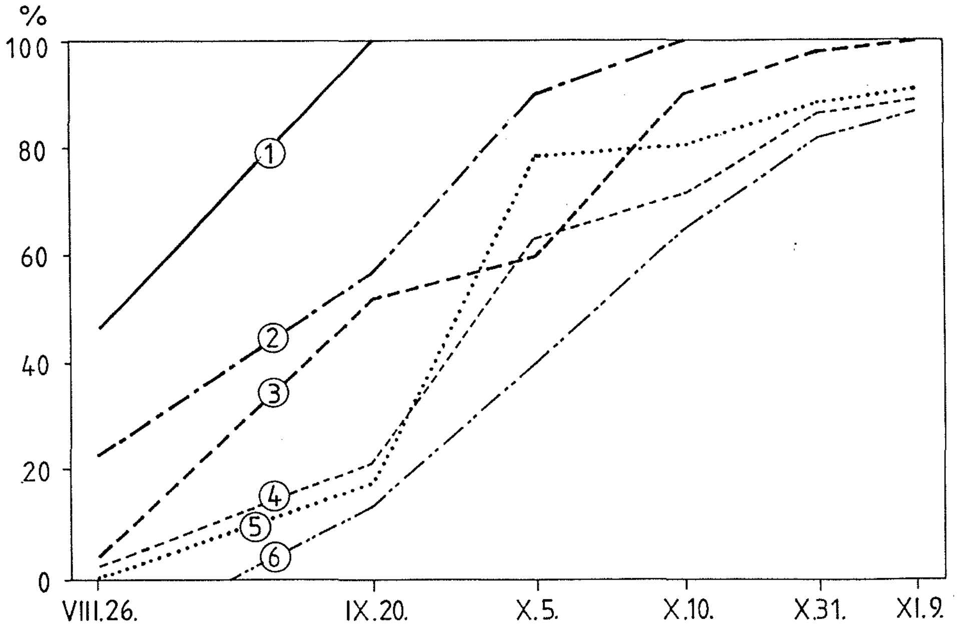
The genetic system of the species plays an important role in maintaining the adaptation lag of populations. Outcrossing pioneer (r-strategist) species distributed over a wide range of environments keep geneflow usually very effective (small seeds, heavy pollen flux). This implies that individual populations will be less perfectly adapted to local conditions. On the other hand, species with less effective geneflow mechanisms (large-size seeds, infrequent flowering) and more dispersed distribution are exposed to drift effects which again will hinder optimization of local adaptation. Thus, observed genetic variation at the population level does not reflect necessarily the effect of local selective forces in a precise way and no clues are available to judge the 'closeness' of adaptedness on the basis of supposed 'undisturbed' or 'natural-state' status of the population.

As a result of their different life history and adaptive genetic variation patterns, herbs and trees are markedly different. Therefore, research findings with agricultural crops and annual plants are usually not directly applicable to trees. For forest trees with large distribution areas and effective geneflow (predominantly conifers) there are enough research results which question the 'closeness' of adaptation. For instance:

- Populations respond relatively weakly to changes in environmental conditions and mostly in linear fashion, and the majority of populations reveal high phenotypic stability and low G × E interactions. This is above all true in the central parts of the distribution ranges. In areas close to the species limits stronger responses and reversed trends may appear.
- The analysis of height growth and yield of different provenances in common garden tests show that very often local indigenous populations are outperformed by others (Mátyás and Yeatman 1992).
- There are certain traits, the adaptive significance of which, cannot be questioned. A highly adaptive one for example is the timing of bud break and bud formation, determining the length of the vegetation cycle. Even in the case of such traits, an overwhelming within population genetic variation can be observed, which may be larger than the differences between means of distant populations (Fig. 1).

Climatic adaptation: an opportunity for setting priorities?

In view of severe budgetary time and space constraints it might be justified to consider whether the priority setting for conservation strategies is rational for certain gene or trait groups. For alleles carrying lethal traits this might seem logical. To judge in general over the 'value' or 'neutral' significance of a gene is risky. A neutral gene might improve fitness in another situation as already mentioned. It has to be remembered, however, that there is an analogy at the level of species. In plant communities the concept of differentiated ecological importance of species (e.g. keystone species) is well established. A similar approach may apply at the level of genes as well.



Legend: 1 = Serebryanskoe (former Soviet Union) N. lat. 59°58'/E. long. 33°30'; 2 = Suprasl (Poland) 53°12'/23°22'; 3 = Spala (Poland) 51°37'/20°12'; 4 = Pornóapáti (Hungary) 47°20'/16°28'; 5 = Groenendaal (Netherlands) 50°40'/4°26'; 6 = Çatacik (Turkey) 40°00'/31°10'

Fig. 1. Advance of budset in Scots pine seedlings between 26·August and 9 November in an International Union of Forest Research Organizations (IUFRO) experiment in Hungary. Note that excluding the northernmost provenance (Nr 1 =Serebryanskoe), the phenologic difference between populations (in vertical direction) is much smaller than the genetic variation within populations (in horizontal direction) (Mátyás 1983, unpublished).

If the concept of maintaining evolutionary potential is accepted as a priority in gene conservation, then traits linked to adaptation have to be considered as highly valuable in an uncertain situation. An adaptive trait is one that has responded to changes in the environment through natural selection (Brandon 1990; Eriksson *et al.* 1993). The intensity of response indicates its importance in the process. To decide which traits contribute to the adaptive ability is not simple; the most conservative approach is an analysis of climatic adaptation within a species' range. Climatic adaptation reflects first of all responses at the population level to changes in macroclimatic and main stress conditions within the distribution range of the species, as well as the change in climate and stress conditions to be considered when designing gene conservation strategies for an unstable future. No doubt there are numerous traits which determine fitness and adaptability but which are not linked to climatic adaptation, such as fertility and seed production. It may be proven, however, that variation of these traits appears mainly at within population level.

Climatic adaptation can be analyzed to clarify whether links between ecological factors and intraspecific genetic variation patterns exist. At the species level, an environmental component is considered an 'ecological factor' if it can be related to the species distribution and abundance pattern. This might be applied at the level of traits and genes as well. The variation pattern of adaptive traits correlates with the pattern of ecological factors. These are usually macroclimatic effects. Similarly, an ecologically significant adaptive gene is one that displays allelic frequency patterns which may be significantly and logically related to the variation in the environment. However, the existence of a correlation is in itself no proof of the causality of the relationship.

The assessment of the effective population size or selective neighbourhoods is of importance for the spatial planning of gene conservation measures, i.e. the size of populations within which genotypic ranking by fitness is more or less the same and geneflow creates gene frequency conditions which are sufficiently uniform to be considered as one approximately homogeneous unit. Again, it is climatic adaptedness that may help to resolve this question if populations sampled in areas with different climatic gradients are investigated.

There are numerous methods to analyze climatic adaptation, such as:

- common garden or reciprocal transplant tests of sampled populations (in forestry provenance tests);
- analysis of the relationship between environmental factors and gene frequencies as well as average population performances;
- comparison of ratios of within and between-population variation of traits;
- analysis of reaction norms, response regressions and G × E interactions.

Analysis of climatic adaptation in widely distributed coniferous species revealed a strongly buffered, robust and weakly differentiated variation. This seems to be in strong contradiction with the fact that in central Europe for instance, forest decline affects nearly all species and only a part of this phenomenon can be attributed to non-climatic effects (diseases, air pollution). If extreme weather conditions in the last decade play an important role, which seems to be the case, then the genetic buffering of populations cannot be so effective.

In fact, when analyzing populations close to the southern distribution limit of the species, calculated reaction norms show that the 'local response' is positioned asymmetrically and populations are well buffered only towards cooler conditions. This has been proven for example jack pine (*Pinus banksiana*) in Ontario (Mátyás and Yeatman 1992) and yellow pine (*Pinus ponderosa*) in California (Mátyás 1995). Towards warmer sites, or in cases of increased drought stress, populations react with instant growth loss (Fig. 2). Close to the northern distribution limits this phenomenon turns around (Persson and Beuker 1995).

Contribution of molecular genetic tests

In certain agricultural crops (wheat, rye, barley) there are examples of non-random distribution of polymorphisms. Some of these may be of adaptive character and correlations between fitness and number of heterozygous loci have been established (Perez de la Vega 1995). It has to be considered, however, that these species are predominantly selfing, which is very uncommon in trees.

In the case of trees, the adaptive value of certain alleles cannot be proven (Hamrick *et al.* 1991; Savolainen 1994). Neither isoenzyme analysis, nor DNA tests have provided convincing markers which could be considered significant in an ecological sense. Some geographic patterns are detectable but their adaptive significance is questionable, as they may be the result of random or migrational effects. For instance, Savolainen and Hedrick (see Savolainen 1994) found no evidence of correlation with fitness traits. Some exceptions are notable, however, Bergmann and Gregorius (1993) found that at one locus of the IDH enzyme the allele with the highest thermal stability displayed the highest frequency southward, along a north-south transect in European fir. Savolainen (1994) was able to show significant rDNA variation at population level for Scots pine (*Pinus sylvestris*) Finnish provenances (Table 1), but there was no detectable clinal trend in the pattern. In oaks, cpDNA analyses for western Europe revealed interpretable patterns but these are most probably the result of migratory effects (Petit *et al.* 1994).

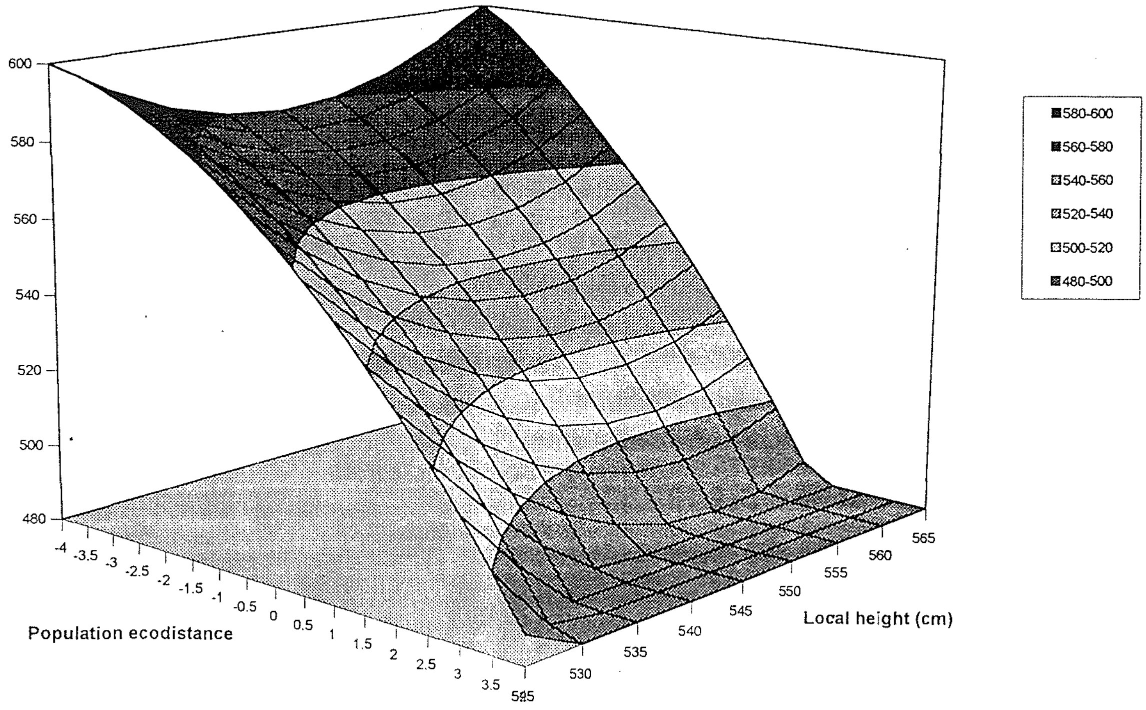


Fig. 2. Response regression model of 45 yellow pine half-sib families in four tests in California. Actual height (vertical axis) is illustrated in function of site potential (height of local provenance) and transfer distance from the location of origin of the source (expressed in average annual temperature differences). It may be observed that growth diminishes rapidly with increasing temperature (Mátyás and Kitzmiller 1995, unpublished).

Compared to the clinal character and relatively high between-population variation observed for adaptive quantitative traits, molecular genetic tests at present contribute little information to understanding the mechanisms and patterns of climatic adaptation. One reason could be the generally neutral character of investigated loci. On the other hand, if natural selection acts on allelic combinations and not on individual alleles, then its detection at a molecular level becomes very complicated.

Thus, molecular genetic analysis, although indispensable, cannot substitute quantitative analyses in field tests. The same applies for tests in greenhouses, controlled environments and even nurseries.

Table 1. Ratio of between-population genetic variation, as a percentage of total, for different trait groups in Scots pine (Savolainen 1994)

Allozymes: 13 loci	2%
rDNA	14%
Budset data	80-90%

Principles to be considered for formulating a conservation strategy

For widely distributed tree species with effective geneflow mechanism, it seems to be proven that adaptation to the local site conditions is not perfect. The size of selective neighbourhoods with approximately homogeneous allelic frequencies seems to be quite large, which implies a relatively strong genetic buffering against changes in environmental conditions. Assuming unchanged conditions for the foreseeable future, the task of gene conservation would not appear to be very pressing.

Regarding expected climate instability and other uncontrollable changes it seems, however, that natural mechanisms of adaptation might not be sufficient to keep pace with expected changes (this may be even more the case for tree species with less effective geneflow mechanisms). It is inevitable to plan active human interference in gene conservation programmes, e.g. selection of stands for regeneration, planned transfer of populations and even conservation breeding measures. Much of this strategy has to be accommodated in the guidelines for the controlled use of forest reproductive material and the silvicultural treatment of species.

Insufficient preconditions for gene and species migration create specific urgent problems for the active conservation and regeneration of populations on the southern fringes of distribution areas. Environmental stress can easily cause extinction of such populations which need special attention in view of their high vulnerability.

Owing to widespread human interference and fragmentation of ranges, the idea of enhancing genetic adaptability to prepare for uncertainties should be introduced into general forestry practice. The most effective means is the preference of phenotypically plastic populations. Plasticity should be regarded as being at least as important a trait as yield in the silviculture and tree improvement of the future. Plasticity differences have been found not only at clonal level but also between populations, therefore, possibilities for improvement do exist (Fig. 3).

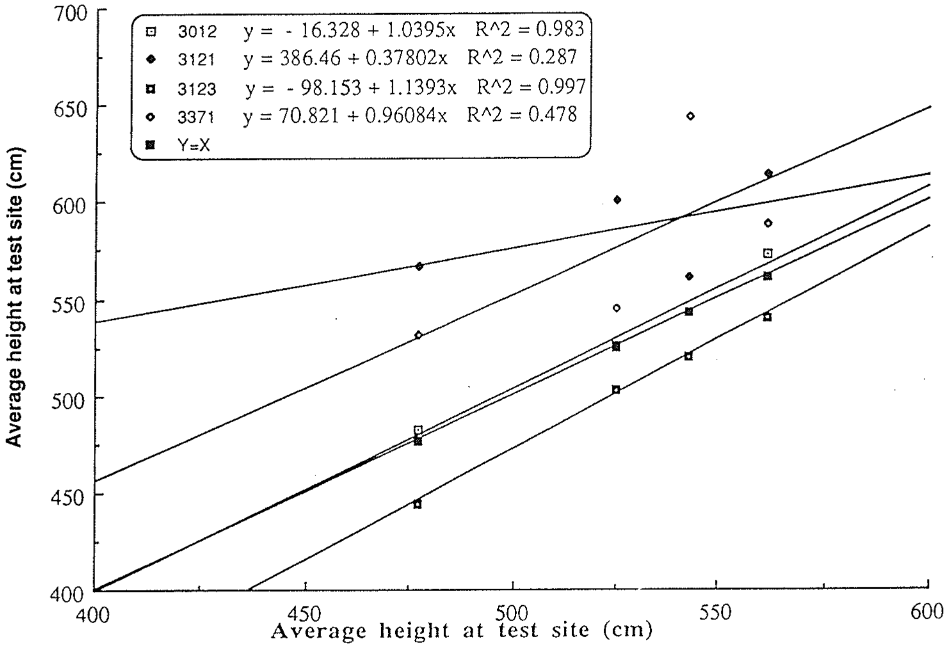


Fig. 3. Phenotypic plasticity in 4 yellow pine (*Pinus ponderosa*) half-sib families in 4 tests in California. Differences are indicated by the changing slope of the equations (Mátyás and Kitzmiller 1995, unpublished).

The colonization of new environments is another problem that has to be analyzed and considered in gene conservation strategies. It will be a special conservation task to select methods to facilitate migration of properly adapted populations on the northern fringes of distribution areas.

The expected environmental instability justifies the setting of priorities in conservation. It is first of all the group of traits reacting to climatic differences within the range, i.e. adaptive traits, which deserve special attention. Geographically structured, variation patterns of adaptive traits should preferably be utilized in conservation decisions. As a single gene rarely rules such patterns, their detection through molecular genetic techniques is less effective. The establishment of field tests and the analysis of quantitative traits cannot be substituted by molecular genetic studies.

Detailed maps of the species' distribution are an indispensable help for the planning, genetic sampling and interpretation of results. Data of ecological character (local climate, occupied niches etc.) are also very useful.

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Genetic control of phenotypic traits with relevance to gene conservation in trees: a survey of methods*

Hans H. Hattemer and Martin Ziehe
Abteilung Forstgenetik und Forstpflanzenzüchtung, Georg-August-Universität Göttingen, 37077 Göttingen, Germany

Introduction

The choice among candidate materials for gene conservation has conventionally been supported by an inventory of genotypes at marker gene loci. This helps to gain information on genetic structures represented in populations and provides some insight into the genetic system of the species or populations in question.

During the past two decades, Gregorius has derived a family of efficient parameters that enable the research worker to condense and characterize aspects of the information contained in genetic structures (see Hattemer *et al.* 1993 for a compilation). Measures of genetic distance and differentiation allow for the comparison of frequency structures. Measures of genetic variation, above all the diversity measures, permit inference on the genotypic variability which a population may exhibit on the basis of the alleles encountered at marker gene loci.

We define a genetic resource as biological material possessing either a particular genetic information or a large number of not too rare genetic variants (Ziehe *et al.* 1989). Biological material may then be declared a genetic resource on the basis of certain genes contained in its genepool with sufficient frequency. However, we cannot reliably evaluate the expression of traits which directly add to the adaptive capacity of a population unless the genotype at structural loci controlling these traits is observed. In view of this difficulty, there is a tendency to infer the adaptive capacity of tree populations solely from their variation at particular enzyme gene loci. Special methods have been derived for measuring not only diversity but also the adaptive capacity of populations (Gregorius 1994, 1995; Ziehe and Gregorius 1996) and considering frequent and rare alleles separately in order to estimate adaptive (operating and latent genetic) capacity (see also Bergmann *et al.* 1990).

In general, the genetic variation of a population is a prerequisite of any process of adaptation. However, future environmental conditions and the contribution of given genetic variants to the fitness of the population under these conditions cannot reliably be predicted. Hence, the number of variants at certain enzyme gene loci and their frequency structure may still be considered to be less specific indicators of adaptability. It may, therefore, be advisable to accompany these genetic data by findings on adaptive phenotypic traits.

Recently Skrøppa (1995) and Eriksson (1995) advocated the integration of phenotypic traits into decisions on genetic resources. Mátyás (1996 and this volume) have emphasized the dominant role of adaptation to the temperature climate in trees. There is no doubt that adaptedness to particular environmental conditions should play a role. As this phenotypic trait varies among populations and between environments it should be described by certain physiological (Larcher 1994) or morphological traits of the trees or populations in different environments. It is imperative to test whether these traits are under genetic control. In a changing world, adaptability is also required.

The role of particular enzyme gene loci in controlling adaptedness has been investigated with regard to pollution stress only in a few instances (Bergmann and Scholz 1989; Müller-Starck 1993 and the literature cited there). In even fewer instances, the physiological effects

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of certain allozymes under pollution stress have been explained (Rothe and Bergmann 1995) or they have at least been plausibly described (Hosius and Bergmann 1993; Bergmann and Hosius 1996). The adaptedness of allozymes to temperature regimes has been analysed by Bergmann and Gregorius (1993).

Apart from special cases, we must confine ourselves to the question of whether the variation of a certain phenotypic trait is based on rigorous genetic control or whether environmental modification prevails. We subsequently shall consider three types of approaches to test whether the variation of traits in a given situation is under genetic control.

Phenotypic differentiation of demes in experiments

Differences among provenances, stands, families or clones of a botanical species (in this paper called demes for the sake of brevity) in their phenotypic trait expression may be attributed to their genetic differentiation. Conclusions that the respective traits must be under genetic control can be reliably drawn only if certain methodical requirements are fulfilled.

The present approach generally cannot yield information on the mode of inheritance, unless the demes are related by descent. Only the comparison of phenotypes of parents and their progeny would permit deductions on the transmission mode of the controlling genetic information and the way this information is expressed. However, this is possible only if the number of controlling gene loci is very small and if environmental modification is minor.

Basic principles

The fundamental requirement for inferring genetic control from differentiation among demes is that the experimental material representing the various demes receives absolutely equal pre-treatment. Furthermore, differentiation can be proven by carefully designed experiments only. The experimental units (in field experiment plots) must be properly randomized in order to establish independence of genetic and environmental effects on phenotypic variation. This is a prerequisite of significance tests and an important item in the evaluation procedure. Every deme must be observed in several experimental units, i.e. the experiments must be replicated. In addition, the experimental units must be arranged in a way that keeps the experimental error low. Efficient means of controlling experimental error are the layout of the experiment in blocks, and shaping the blocks and plots to minimize noise. Needless to say, during the course of the experiment any effects acting on several but not all demes should be avoided.

The evaluation of relevant data is usually done by statistical tests on means of traits following an analysis of variance. This procedure neglects one genetically important item. A difference between the means of demes tends to be larger if the distributions within those demes overlap only a little or not at all. A difference tends to be smaller the more the distributions overlap. However, neither the size of a given difference nor its significance indicate whether the compared distributions do overlap or not. This is helped by the concept of genetic distance. According to Gregorius and Roberds (1986), the genetic distance d_0 (Gregorius 1974) measures the proportion of genetic elements not shared by two demes. This may also be interpreted as the minimum proportion of substitutions required in order to change the distribution within one deme into the distribution within the other one. Verga (1995) applied an extension of the genetic distance to metric traits. This permits a comparison among demes on the basis of their distributions. The overlap between the two distributions ($1- d_0$) allows estimation of the proportion of phenotypes in one deme that is also produced by the other one under the present conditions. The complement (d_0) of the overlap may also be interpreted in terms of the proportion of one deme that had to be substituted in order to produce the same distribution as in the other deme. In view of the heavy reduction of population size of trees with increasing age, it makes more sense to concentrate on this aspect of the differentiation of demes rather than rely on the unspecified difference between their location measures.

Field experiments

Most of our experience on genetically conditioned phenotypic variation has been accumulated by field experiments. Unfortunately, many years after the first publication of R.A. Fisher's Statistical Methods in 1925, numerous long-term field plantations comprising different demes of a species such as provenances have been established without giving much thought to design. These plantations are nevertheless considered as experiments but can only be used to demonstrate major phenotypic differences between genetically characterized demes that otherwise become indistinct by the range of environmental effects.

Results from observations in a particular environmental situation may prove genetic control of phenotypic traits but nevertheless be of restricted or no relevance for predicting phenotypic reactions under different environmental conditions. Replication of an experiment with the same set of demes in different locations has long been advised and practised in provenance research. In general, it is not specified whether test sites should be located where they happen to be available or where they represent specific environmental conditions.

To most foresters, the observation of growth and adaptedness makes sense only if the demes were exposed to field conditions. However, the conditions may be considered to be highly specialized in that demes are compared in an even-aged pure stand planted at regular, preferably wide, spacing on a homogeneous bare site. All this is somewhat different from a forest environment and consequently of reduced predictive value for naturally regenerated populations.

The length of the observation period of an experiment is restricted depending on plot size. In most experiments, only such traits may be assessed that are expressed at an early age. Planting an experiment in order to observe a certain trait consumes time until the trait is expressed. This is a serious obstacle for progress in research on long-lived woody plants. Ewald *et al.* (1996) estimate that curly birches cannot be identified before the age of nine years. Teissier du Cros *et al.* (1980) found that Krahl-Urban's (1962) observations on the incidence of spiral grain could be considered reliable at the age of 10 years at the earliest. On the other side, phenological traits may be assessed on several occasions as early as the nursery age. The observation period of experiments may be relatively short if compared with the life span of trees. Therefore, not all extremes of temperature, rainfall and windspeed can be expected to be realized. Last but not least, the judgement of the behaviour of the tested demes is retrospective and under environmental change that does not necessarily apply to the future. Much data of this type has been accumulated by forest tree breeders.

Ecophysiological tests in the laboratory

The expression of many physiological traits can be measured only in a controlled environment. The test environment in the laboratory or climate chamber is even more artificial and, therefore, possibly less representative of the natural environment of trees than that in field experiments. However, certain values of crucial environmental factors can be simulated. This can be done regardless of whether these conditions have occurred in the past or are only predicted to occur in future. The view may be thus prospective. Examples are the plasticity of reaction to high temperatures, fumigation tests in open-top chambers, lab tests on frost resistance (Larsen *et al.* 1988). Last but not least, adaptedness in the sense of Larcher (1994) is a trait of this category.

Parent-offspring relationships

Similarity between parents and their progeny is extensively exploited in breeding theory. The enormous breeding progress made by early man was hardly based on comparison tests but rather on the experience that a plant or animal with desirable properties gave rise to progeny with the same or even better value for use.

The similarity between parent and progeny must be based on inheritance and implies genetic control. The covariance between parent and offspring used in estimating heritability, therefore, has a stronger conceptual basis than the covariance between sibs. However, only little can be said about the mode of inheritance.

Regression of offspring on parent can be tested in stands and their bulked progeny; this has been investigated in very few instances in the past. It can also be studied in seed parents and their progeny (from natural regeneration) in the stand or seed parents and their progeny growing in experiments. In the absence of major self-fertilization, the similarity is then primarily based on the female gamete contributions of the progeny.

Collecting seed from the trees and raising plant material elsewhere for establishing an experiment possesses the advantage of stochastic independence of the environment between parents and their progeny but costs time. Also, the environment is artificial to some degree. In field studies, the seed parents and their progenies in the forest are compared in the same local environment where the process of reproduction took place. The time efficiency is much increased, since trees can be used that are already there. However, even if the seed chorology admits the attribution of young trees to only one seed parent, it is essential to check the descent of the progeny by genetic markers.

Finally, pairs of mated parents and their progeny after free pollination can be used. This type of experiment permits statements on extranuclear and dominance effects of nuclear genes if reciprocal crosses were made. In trees, clonal seed orchards are useful for producing those crosses for reasons of cost efficiency. Examples are tests on wood density (Persson 1972) and spiral grain (Krahl-Urban 1961, 1962; Teissier du Cros *et al.* 1980).

Forked stems in beech may serve as a further example. This condition has economic implications but is scarcely adaptive. It is still relevant in present conservation practice, since populations with a large proportion of forked trees are hardly given preference if a choice can be made. In beech stands under natural regeneration, Krahl-Urban (1952/53, 1962) has demonstrated higher frequencies of forked saplings around forked adult trees. R. Müller-Starck (1996) estimated a maximum seed transport distance of 20 m with her observations. Given that distances of seed migration are rather short in beech stands, the saplings are expected to be clustered around their seed parent, although this does not mean that all saplings under a tree have this tree as their common seed parent. Following Krahl-Urban's interpretation of the mode of inheritance, clustered occurrence of forks would then mean either dominance or extranuclear inheritance.

According to Merzeau *et al.* (1994) and G. Müller-Starck (1993), the proportion of offspring arising from self-fertilization is rather low in beech. R. Müller-Starck (1996) in her observations of adult trees and beechnuts found the distance of joint seed and pollen transport to amount to a maximum of about 50 m in a closed stand. In conjunction with the indirect evidence of a certain proportion of cross-fertilization among related individuals, as presented by Merzeau *et al.* (1994), clumps of forked trees could then be explained by local condensation of certain genes that are not necessarily dominant. All this is admittedly somewhat speculative, but many foresters still claim that even in adult stands forks occur in clumps. This condition was studied by Turok (1996) using another approach (see next section). The supposed family structure in beech stands has still to be shown by an appropriate combination of genetic markers.

In trees, parents and progeny usually differ much in age. Yet the study of parents and their progeny is a useful approach to the study of phenological traits with their relative ontogenetic stability.

Pair comparisons in the field

It may not be directly testable whether an observed phenotypic variation within a stand has a substantial genetic component. If genotypes and environmental situations are associated, the variation of phenotypic trait expressions may be controlled uniquely by environmental conditions and genetic patterns could also be correlated with phenotypic patterns. Hence, a precondition for reliable conclusions is stochastic independence between genotypes and environmental situations. This is safely fulfilled in planted stands but not necessarily so in stands which originated from natural regeneration. However, it may be indirectly tested whether phenotypic differences are likely to have a genetic component. For this purpose, pairs of trees can be selected in the forest depending on the expression of a phenotypic trait. At first a tree with phenotype *X* is found, then its nearest neighbour with contrasting phenotype *Y* is selected. This sampling procedure is supposed to ensure local stochastic independence between genotype and environmental situation (Gregorius 1989). It is essential that the partners could have emerged from the same seed pool. Hence, the contrasting phenotypes should be located within usual seed transport distances from a single potential seed parent.

After genotyping all pairs of trees at a set of marker gene loci, a significance test of homogeneity between the genetic structure of phenotypes *X* and that of phenotypes *Y* is undertaken (Fig. 1). If the null hypothesis is rejected, phenotypes *X* and *Y* are associated with different genotypes and the trait must be under genetic control. However, gene loci with significant differences are not necessarily directly involved in controlling this trait; they may be only stochastically associated with controlling gene loci. Such stochastic associations can be easily produced by mixed selfing and random mating resulting in a correlation of heterozygosity and a correlation of homozygosity over the gene loci. Also small reproduction effective numbers can induce stochastic associations.

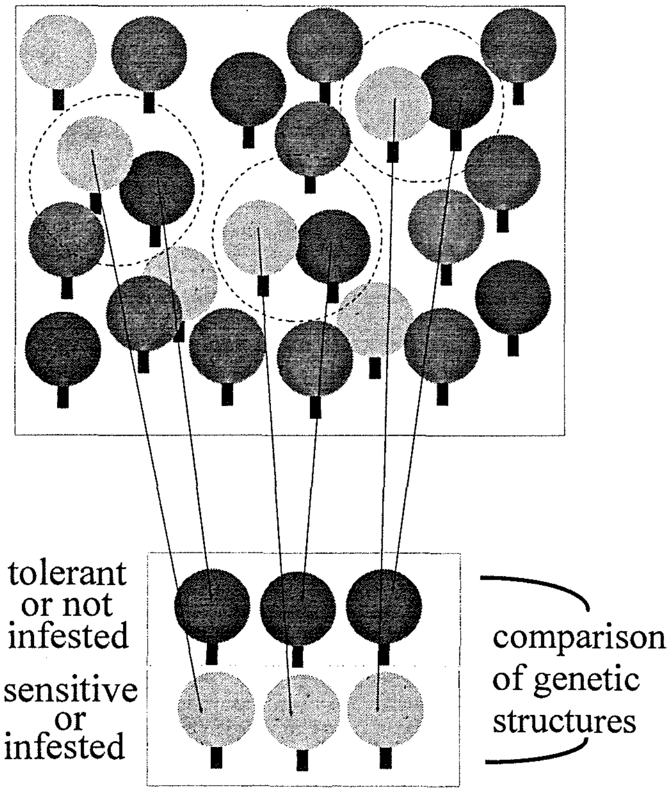

Fig. 1. Illustration of the method of pairwise sampling (modified from Ziehe and Gregorius 1996).

Genetic distances quantify the difference in genetic structure between the phenotypic groups. Reliable genetic distances require sufficiently large sample size, in particular if high genetic diversity is encountered at the observed gene loci. On the other hand, gene loci stochastically associated with controlling loci are more likely to show substantial distances if they carry a larger amount of variation. Consider for example an associated minor polymorphism with one very frequent and one very rare allele and consequently a low frequency of heterozygotes. Only a small genetic distance between the phenotypic groups can be expected if heterozygosity is slightly correlated over the gene loci and is essential at the controlling loci for the observed trait expressions.

In some past applications of this method, pairs of neighbour trees were chosen only for their contrasting phenotypes. This is not quite the same as selecting the partners according to the procedure devised by Gregorius (1989). However, with an empirical check on the results of the two approaches, Konnert (1992) found only a little difference in her study of immission tolerance in white fir.

The application of this indirect method cannot possibly yield any information on the mode of inheritance. However, the method possesses current importance. The genetic control of traits in trees of any age can efficiently be tested in the forest environment after the stand has been thinned repeatedly. The method has essential practical advantages, since it may be applied also to non-reproducing trees and to trees of any age.

If carriers of contrasting phenotypes *X* and *Y* tend to show similar frequency deviations in different populations, we may call the genetic differences consistent. Then either stochastic associations with the controlling gene loci are of a similar type or the surveyed marker gene locus is involved in the genetic control of the trait. The evidence for the latter can substantially increase for predominantly outcrossing species with increasing number of populations having different genetic background.

This method has been applied in studies of genetically determined tolerance of beech trees (*Fagus sylvatica* L.) to immissions. Herewith, phenotypic classification followed Roloff (1985) with class 0 for tolerant and class 3 for sensitive trees. From 5 populations located under different environmental conditions, Müller-Starck (1993) investigated samples of between 43 and 53 beech pairs per population at 15 enzyme gene loci and proved significant genetic differences between tolerant and sensitive beech trees at some of these loci. Ziehe and Gregorius (1996) as well as G. Müller-Starck and Ziehe (submitted) used radial diagrams (Fig. 2) in order to illustrate genotypic and allelic distances at particular sites as compared to differences for the bulked material of all samples. Figure 2 demonstrates the results for the Harz sample. Here gene loci PGM-A, LAP-A, GDH-A, and SKDH-A show genotypic distances of more than 20% between tolerant and sensitive trees. Moreover, for the first three of the mentioned enzyme gene loci, the genotypic distances over all samples are also substantially larger and located between 10% and 20%. It indicates that for these gene loci, the same genotypes appear to be more frequent among the tolerant beech trees as compared to the sensitive trees of the samples, independently of special site conditions of the different populations. This does not at all hold for SKDH-A, which obviously responds differently over the samples. Moreover, since the overall genetic distance is small, other populations than the Harz stand must show opposite behaviour of genotypes, more frequently observed among tolerant beeches, in order to equalize the structures among tolerant and sensitive subpopulations. This is hardly explained by different stochastic associations to the genetic background but is more likely attributable to different environmental situations. Hence, such a diagram is expected to also characterize the environmental factors of the respective site and to offer conclusions about the variation patterns of environmental conditions involved in controlling the observed phenotypic trait expressions.

Gregorius (1995) derived a method which is appropriate to estimate genetic adaptational capacities, i.e. proportions of individuals which are adapted or able to adapt because of their genetic constitution and not because those trees did not experience stress conditions within their microenvironment. Ziehe and Gregorius (1996) applied this method to the above mentioned data from Müller-Starck (1993) and discussed the procedure and the results within the context of gene conservation. According to this, it can be taken from Figure 2 that the estimated adaptational capacity of the tolerant subpopulation is larger than L.

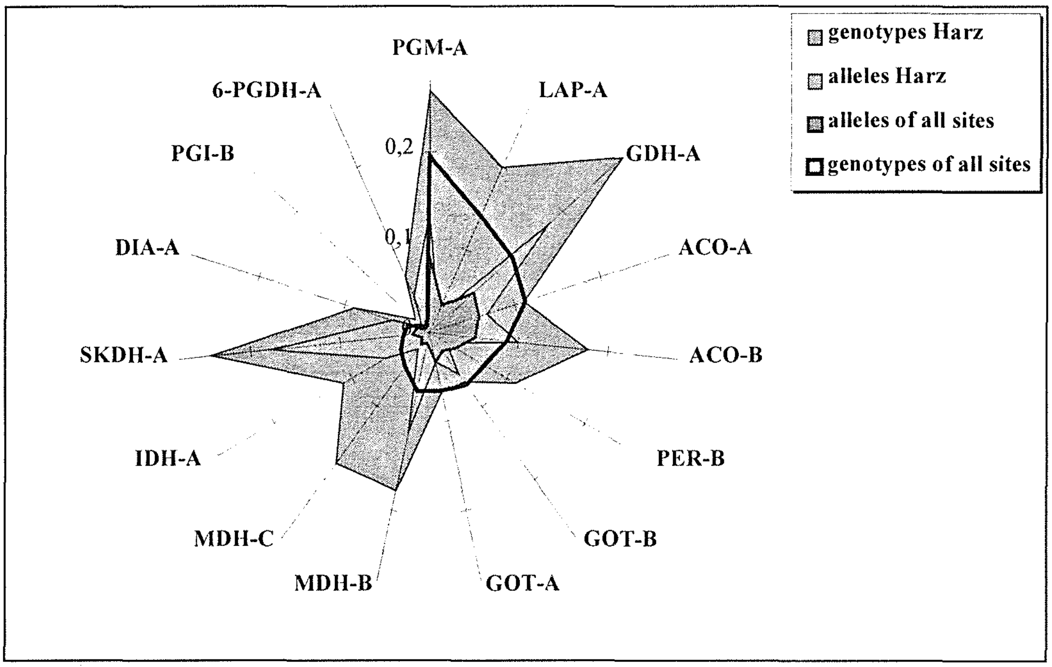

Fig. 2. Allelic and genotypic distances between sensitive and tolerant adult beech trees within the Harz stand and for all five populations at different sites.

The method of pair comparison has also been applied in an investigation of the resistance of European beech (*Fagus sylvatica* L.) to beech scale (*Cryptococcus fagisuga* Lind.) by Gora *et al.* (1994). This is of particular relevance, because beech scale infestation is related to infection with *Nectria* and beech bark disease. Genotypic structures at the enzyme gene locus IDH-A turned out to differ significantly between trees infested and those not or only slightly infested by this insect. Moreover, in samples from different locations (Solling hills with an additional stand sample on one hand and on the other hand a beech stand of the forest district of Bovenden close to Göttingen with different environmental conditions), the heterozygotes always proved to be substantially more frequent among the infested beech trees as compared to the slightly or not infested trees (Fig. 3). In combination with the stand structures, this indicates a genetic predisposition for infestation by *Cryptococcus fagisuga*. However, the physiological role which IDH-A variants may play for resistance is not well known.

In a study on the genetic control of forked stems in young beech, Hussendörfer *et al.* (1996b) did not find a significant genic distance between forked and unforked stems, although allelic distances at several gene loci were rather pronounced. Turok (1996) reported similar findings in an old stand with a clear morphological distinction between the two morphs. In this stand, forked stems occurred in clumps, even though during repeated selective thinning many of the forked stems must have been removed. Neither the genetic distance at the observed enzyme gene loci between forked stems and their unforked neighbours nor those between the respective demes in general were significant. However, in both studies, the forked stems possessed statistically significant higher genic multiplicity. Higher fixation coefficients among the forked trees could hardly be sufficiently explained by the assumption that these trees may have originated from self fertilization. In beech, a substantial effect of large self-fertilization has been refuted in several independent studies.

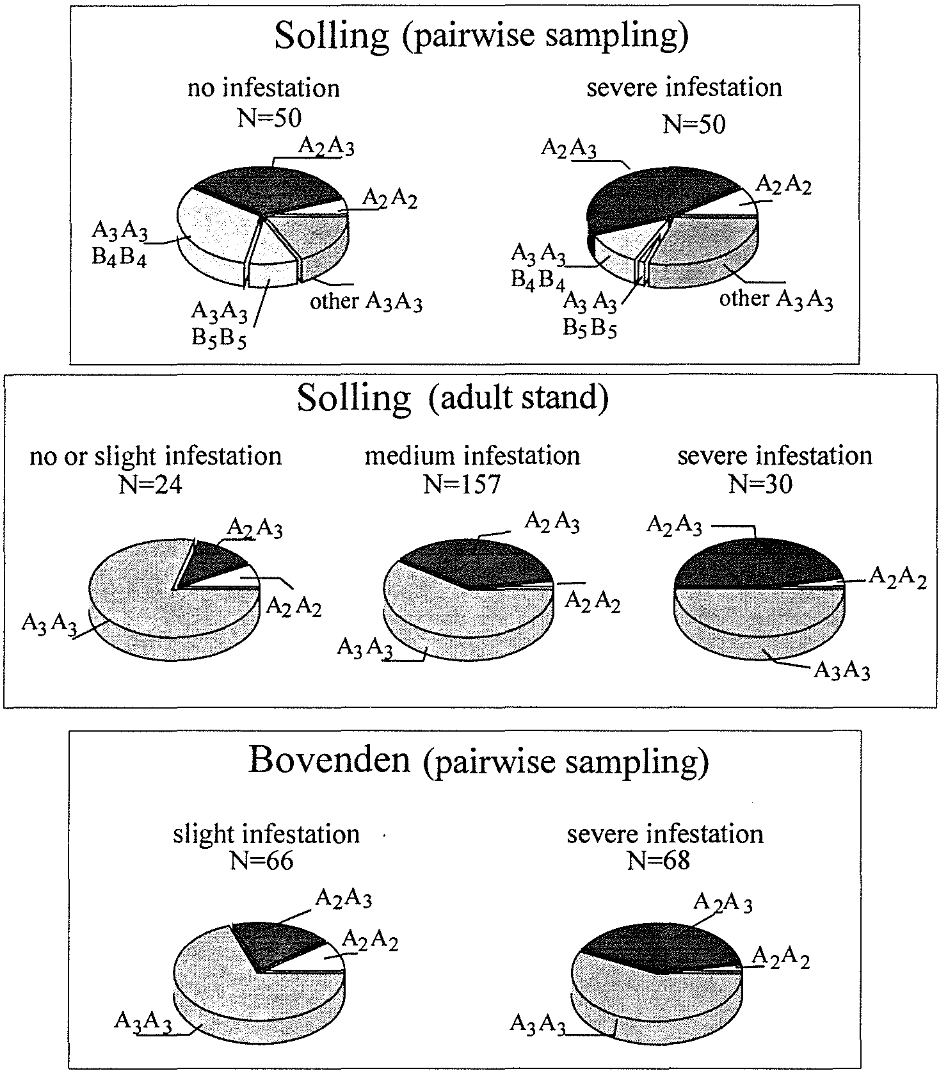

Fig. 3. Comparison of genotypic structures at the gene locus IDH-A between samples with different degrees of infestation by beech scale (after Gora *et al.* 1994; Krabel *et al.* 1995; Petercord and Krabel, submitted). For pairwise sampling in the Solling stand, genotypes B_4B_4 and B_5B_5 refer to the gene locus PER-B.

The extension of pair comparisons to traits with more than two expressions is straightforward. It should be clear that pair comparisons are important in studying the adaptedness and other physiological traits of tree populations and the variation of these traits within those populations.

Response to selection

Johannsen did the first experimental studies on this phenomenon during the first decade of the 20th century. He proved that the seed weight of bean lines with single-seed descent did not respond to artificial selection within lines because of the lack of genetic variation.

Johannsen wanted to show that genetic improvement has genetic variation as a precondition. He did not want to resolve the genetic basis of the observed pronounced variation between lines of this predominantly self-fertilizing species.

Artificial selection for a phenotypic trait continued over generations is a clumsy method for the study of its genetic control in long-lived woody plants. However, if a population responds to selection for a given phenotypic trait with changed genetic structure at a (marker) gene locus, then the trait must be genetically controlled.

If the response of several populations to similar selection regimes is consistent, then the observed marker gene locus must be considered either to be among the gene loci that control the trait under selection or to be stochastically associated with such gene loci. If the considered trait is the viability, then the controlling gene loci must be considered to be adaptive.

From data of a larger experiment with beech seedlings, Müller-Starck (1993) determined viabilities of different genotypes at several enzyme gene loci. Survival was estimated for the ontogenetic phase from pre-germinated beechnuts till juvenile beech at the age of two. Figure 4 illustrates results for DIA-A (MNR-A). The response is consistent over different seed material and field conditions in favour of the homozygote A_2A_2. The frequency of the second homozygote was too low to allow estimation of reliable survival parameters. It is unclear whether this reflects a response to new environmental (stress) conditions not experienced previously, or the polymorphism has been maintained by counterbalancing selection in later ontogenetic stages. Ongoing selection of the present type would certainly increase the danger of losing genetic variation.

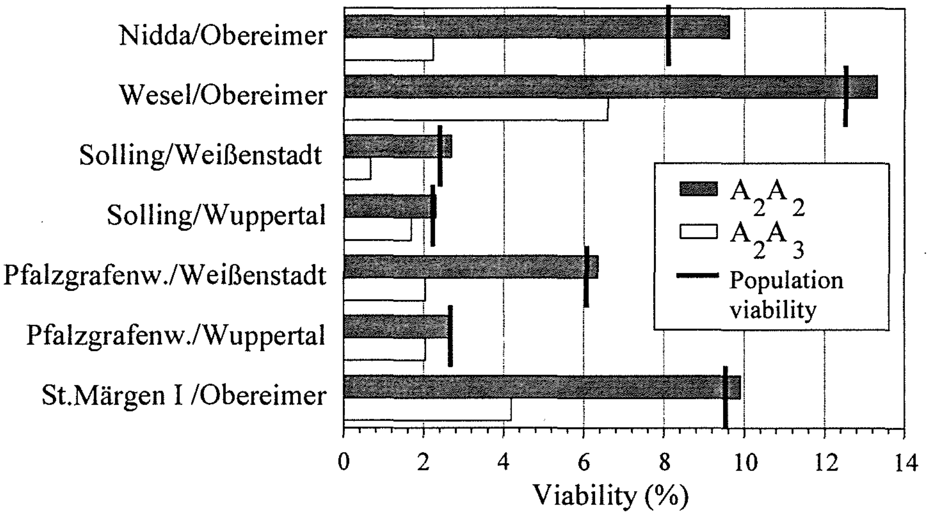

Fig. 4. Viabilities (in %) of the different genotypes of beech seedlings at the enzyme gene locus DIA-A (MNR-A). Vertical bars indicate the mean (population) viabilities of the complete samples. Seed samples originated from Nidda, Wesel, Solling, Pfalzgrafenweiler, and St. Märgen. Seedlings experienced field conditions on sites at Obereimer (Arnsberg, Sauerland), Weißenstadt (Fichtelgebirge) and Wuppertal.

Conclusions

The declaration of genetic resources must take into account the expression of phenotypic traits such as tolerance to environmental pollution. It may be expected that adaptedness to such an environmental factor that has not occurred during evolution will persist. Temperature regimes have changed repeatedly and drastically during evolution, but a rise in global temperature has been predicted to last a long time with substantial spatial heterogeneity. The implied changes in biocoenoses can hardly be foreseen. All we can do is to attempt to estimate adaptedness and adaptive potential (Degen 1996), and adaptive capacities (Gregorius 1995; Ziehe and Gregorius 1996).

Marker gene loci have several important applications in conservation:

- The measurement of variation and differentiation is basic to the evaluation of candidate materials.
- The analysis of the genetic system of a population allows a decision on whether it is operationally eligible for conservation. For conservation both *ex situ* and *in situ* it is essential to gain some insight into the degree of reproductive isolation of a resource. This isolation is also crucial in deciding whether a resource is large enough or whether it comprises but a restricted number of adult trees that are in mating contact with other populations, boosting the effective size of the resource in question. The analysis of allelic profiles of marker gene loci is helpful in this respect.
- Inference on the descent of populations may be important. Several examples about private alleles of populations or races have been presented (Bergmann 1984 in Norway spruce, Konnert and Bergmann 1995 in white fir).
- More often, the presence or absence of certain marker genes can characterize allopatric species. After the recent establishment of mating contact, it is possible to make statements about species purity (Rajora 1989, Vornam *et al.* 1994 for examples in *Populus*; Häcker and Bergmann 1991 for an example in *Larix*; and Hussendörfer *et al.* 1996a for an example in *Juglans*).
- Marker gene loci are helpful in testing the genetic control of traits. This applies to both the reconstruction of descent of individuals and their employment in pair comparisons.

The last item in this list is not the least important. Making statements on the variation of certain traits, and even more including these traits in the declaration of genetic resources, is misleading as long as it is not known whether and how these traits are under genetic control.

It is difficult to make gross statements on the relative importance of genotype and environment for character expression. It is perhaps not even meaningful. This lesson can also be learned from the enormous variation among published estimates of heritability of a trait in a botanical species. Besides the adaptive value of traits, it is the rigidity of its genetic control that should matter in the declaration of genetic resources. Helpful in this respect is the concept of reaction norms (Gallo *et al.* 1996). Ruetz *et al.* (1996) condensed the declaration of genetic resources to a rule "Choose the most adaptable among sufficiently adapted materials".

Acknowledgement

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Boreal forests: gene conservation in relation to nature protection and forest management

Veikko Koski
Finnish Forest Research Institute, 01301 Vantaa, Finland

Introduction

The issue of genetic diversity of forest trees often raises discussions and dispute. In this connection, terms such as nature reserves, national parks, primeval forests, tree breeding and cultivated forests are often confused; the scales of spatial and temporal dimensions are often obscure; and the natural dynamics of forests ignored. I find it useful to think of the role of gene conservation in the real world. Is it equal to nature protection? Do we need anything apart from a large amount of protected areas or, do we need gene conservation as a separate activity?

One way to arrange the concepts is to consider the different types of populations. Population is used here as a general term, not in its strict genetic meaning. Forest populations can be classified into three main categories:

i) unmanaged natural populations;
ii) managed natural populations;
iii) managed artificial populations.

A more detailed characterization of populations in the following sections illustrates the role of gene conservation. Table 1 gives some relevant characteristics of several types of populations. The list is not exhaustive, but most cases fit one of these types.

Table 1. Characteristics of forest population types

Type	Physical size	Genetic variation	Approach	
			Operational	Genetic
Nature protected area	large	large	passive	static
In situ gene reserve	small	large	active	dynamic
Natural timber production stand	very large	declining	variable	indifferent
Breeding and research population	very small	large, stratified	active	dynamic
Seed production population	very small	moderate or small	active	selective
Artificial timber production stand	very large	uncertain	active	ambivalent

Nature protected areas

Various types of protected areas, such as nature reserves, nature parks and national parks often include large forest areas and can thus be considered as a measure of *in situ* conservation. A large protected area most likely contains broad natural genetic variation. The main purpose of nature protected areas is ecosystem conservation often to preserve a specific spectacular landscape and/or to provide a habitat for endangered animal species. As nature reserves are inherently unmanaged by law, the operational approach is passive and the genetic approach static. Nature reserves tend to be located on specific sites, which are marginal from the point of view of forestry. A serious restriction is the limited availability of the actual genetic resources from nature protected areas. The frequent predominance of old growth forest in such areas is also of concern because of uncertain regeneration. Protected areas have often been the subjects of emotional discussions and restrictions may become still more stringent.

In situ gene reserves

In situ gene reserves are characterized as managed forests, even though they are made up of predominantly natural populations. Their size ought to be large enough to contain the broadest possible genetic variation within a breeding zone. However, the area needed is much smaller than that usually covered by a nature park. The crucial difference between managed gene reserves and nature protected areas (reserves) is the pattern of regeneration. Planned stepwise regeneration leads to age-class distribution, which is more resistant to various damaging factors than one very old age class. If environmental factors change in the long term, then younger parts of a gene reserve population will have more potential to adapt and the genetic resources of managed stands are available. Collecting reproductive material does not damage the forest, nor does it change gene frequencies. The application of ordinary, locally adapted, silvicultural measures include logging and ensures the management of gene reserves as a part of normal forestry practice.

Natural populations managed and utilized for timber production

The proportion of indigenous stands within managed forests varies considerably from one country to another. In the boreal zone most commercial logging is carried out in naturally regenerated forests. The future existence and the biological diversity of managed forests is the main concern of many environmentalists. In the Nordic countries, natural regeneration has been quite common and has become more popular in recent years. In Finland, for instance, 75% of the total forest area is of natural origin, and the share will most likely remain at over 50% in the future.

On the other hand, there is a real threat that short-sighted exploitation or the adverse effects of environmental changes, including changes in land use, will reduce the genetic variation in natural populations managed and utilized for timber production. The demand for valuable timber puts a high economic pressure on remaining natural populations. Modern technologies make logging and transportation possible in locations that used to be inaccessible. Improper regeneration may convert the area to another species or introduce a provenance with unknown or undesired properties.

Breeding and research populations

Long-term breeding populations are established in order to maintain broad genetic variation for future breeding cycles. Such populations consist of selected or tested origins and genotypes. Several kinds of experimental plantations, such as provenance trials and progeny tests, can be referred to as research populations. Both breeding and research populations are intensively managed artificial populations. The origin and often the pedigree of entries is well documented. Breeding populations may be subdivided into lines, into main and core parts, or into small multiple populations. As regeneration is planned to take place by means of controlled crossings and progeny testing, including selection, the number of (unrelated) trees per generation has to be rather small and ranges from 20 to 500 in various models. Sophisticated breeding applications are certainly effective for achieving the breeding objectives, but their benefits as regards long-term conservation of genetic resources are often uncertain. Firstly, the founder material has always been selected for economically important traits and the number of genotypes is rather low. Secondly, controlled crossings with any mating design are more difficult in the field than in theory. Labour costs and safety regulations may also become more severe. Thirdly, the breeding lines will diverge from natural populations and they will be significantly stratified.

Seed production populations

Seed production populations are usually equivalent to seed orchards. First generation multiclonal seed orchards consist of tens of clones and unexpectedly large background pollination still increases the genetic diversity of the seed crop. The area of seed orchards is very small, but they have a large-scale impact on the genetic composition of planted forests. It should be self-evident that each seed orchard has a limited utilization area and life span. First generation seed orchards will be replaced by more advanced seed orchards, that will probably contain fewer parent genotypes. The reduction in parent tree numbers is a necessity, if an additional genetic gain is aimed at. On the other hand, it is to be noticed that each seed orchard is a 'blind end'. An old seed orchard may be abandoned and there is no 'second-generation orchard' arising from natural regeneration. Next generation seed orchards will be established anew, which means that they do not aim at conserving genetic resources in the long term.

Artificial populations managed and utilized for timber production

Planted timber production forests represent an extremely heterogeneous category. They may originate from local bulk of seed from indigenous species, from genetically improved seed, be of foreign provenance or from seed of introduced species. Consequently, the overall genetic diversity can be uncertain. Another aspect is that it is rare for the origin and pedigree to be known. The genetic variation at stand level can be narrow; in extreme cases one single clone. In the case of indigenous species, wild seedlings from partial natural regeneration grow amongst planted ones. When trees were considered in the same way as crop plants, uniformity rather than heterogeneity was thought desirable at the stand level. More recently it is believed that every stand should contain the broadest possible variation. It is to be hoped that in future the co-existence of various kinds of stands will be accepted.

Diversity is maintained by means of species composition, mosaic structure of various genetic combinations and the age-class distribution. With an increasing proportion of intensive plantation forestry, the genetic structure of artificial forests will increasingly deviate from that of natural populations. It could be expected that artificial forests, managed and utilized for timber production, become the largest type of forest in many countries. National and international rules should help to ensure that there is sufficient genetic variation within and among populations. In spite of their large extent they are not a measure of gene conservation. The rules of commercial activities overtake the biologically ideal status of such forests. In my opinion there is no need to put the 'full genetic load' on commercial timber production forests if gene conservation is arranged as a separate activity.

Gene conservation and forest regeneration in boreal forests

Lennart Ackzell
National Board of Forestry, 55183 Jönköping, Sweden

Introduction

The National Board of Forestry has the overall responsibility for management of forest genetic resources in Sweden. The present contribution aims to clarify some practical aspects of gene conservation and forest regeneration from the point of view of a national authority. To develop strategies and establish regulations could be described as a 'bakery', where science, politicians, NGOs, nature conservation and practical forestry provide the inputs. The outcome depends upon the ingredients and the skill in the 'baking process'.

Legal and conceptual framework

In the 1970s, the astonishing news that a search for the origins of wheat was underway by agricultural breeders in the Middle East had a profound effect on gene conservation in forestry. This was one piece in the information 'puzzle' that led to an increased and widespread awareness of the problems related to diversity in ecosystems. Threats were recognized and linked to the uncertain and unpredictable changes in the environment caused by human activities. The public started asking questions such as, "Will we be grilled by UV radiation? Will the sea level rise above my house?". Specific sections of society, among which forestry, then took up this general concern. As one of the outcomes, gene conservatio in forestry became a concept.

The arguments of threats or gains are two of the major driving forces of tendencies in society. For example, the possible threat to society of environmental changes may serve as a catalyst and can be used to encourage action by politicians. Among scientists it is a well-known method of obtaining funding. In a way science often catalyzes these trends. Another example is the involvement of NGOs in nature conservation. Do they see the future positive possibilities or the risks?

Forest management imposes human demands on nature. The evolution of nature is big and important. If we for a moment bring in some dimensions of time, a new ice age, which will cover Sweden with ice, is expected in 7000 to 8000 years. Looked at in that perspective, global warming is a small jump on the curve in a long trend. Global warming is indeed a big concern now, but — to be provocative — we should not overemphasize our years among the billions involved in evolution.

Practical implications

Forestry acts upon nature. First of all, the way a forest functions (and its goods and benefits) in serving human needs is a result of, and depends upon, natural processes both biotic and abiotic. Secondly, in the case of many countries, forests *are* nature and we do not look upon forest trees and their stands as a crop. Therefore, forest industries' raw material is produced in forests that have many functions other than timber production. But it is important to bear in mind that nature is not planned but random and very dynamic.

When we now focus on forest regeneration, the reason is that regeneration plays an important role in maintaining, or even manipulating our forests. Hence, to counteract threats, which may be caused by human intervention in nature, forest operations need to be carefully studied, regulated and managed.

Seed sources

The basic sources are seed stands (source identified by the location coordinates), selected seed stands, seed orchards and clonal material.

European Union (EU) regulations foresee the "use of genetically superior reproductive material to substantially increase production". Hence, in the EU regulations for trade, the normal seed stand that has not been selected is *per se* not accepted. A nursery is consequently not allowed to market local seed unless it is selected. It should not be argued here whether local seed sources are best (it also depends on objectives), but whether an option is given for a plant producer to sell local seed. This is strongly demanded by Sweden and Finland within the EU. Among other aspects, it would increase the basis of available seed sources, promote a diverse use of forest reproductive material and, hence, add to gene conservation.

Concerning the category of selected seed stands, the impact of phenotypic selection has sometimes been overemphasized. Selection of individuals has demonstrated gains at the level of 6% and selection of stands can hardly be more effective. However, as long as these selected stands are not too few, or not too small, there is little to criticize from the viewpoint of gene conservation. There may be a greater concern with regard to rare species as selected seed stands are reduced in number.

Seed orchard regulations need to be more specific. Scots pine and Norway spruce are outcrossing wind-pollinated species where the mode of pollination in seed orchards is very important for the existing levels of diversity and inbreeding. Breeders have found that seed orchards with only two parents show 75% of the phenotypic variation as compared to a seed orchard with many clones. Research on the number of effective clones in seed orchards indicate 10-12 clones according to work carried out by Dag Lindgren (Sweden) and Erik Kjaer (Denmark). In order to obtain the actual required number of clones, this number should be doubled or tripled. The use in seed orchards of 20-30 clones, evenly distributed, should be fairly sufficient. We should be careful to accept a lower number of clones planted in big areas.

In conifers the mitochondria genome is maternally inherited. The number of mothers should, therefore, not be disregarded even if open pollination is the usual practice. Similarly, a limited number of fathers would have an impact on the chloroplast DNA that is important for photosynthesis and is paternally inherited in conifers.

The extreme use of clonal material presents obvious risks. Swedish regulations in this area are based on the possibility of leaving 'clonal' forests to regenerate naturally. We claim mixtures of at least 29 clones. In Sweden clonal forestry is used to a very limited extent.

The Swedish long-term breeding programme for conifers is based on the Multiple Breeding Population System. There are currently around 20 populations with 50 individuals each for both pine and spruce. This provides a good basis to maintain alleles with at least 1% frequency. However, there are population geneticists who argue that neglecting rare alleles in breeding may mean the same as denying the obvious impact of mutations in evolution.

Regeneration methods

The tendency of using natural regeneration in Sweden has increased over the past ten years. The experience of several decades has provided the knowledge to know where natural regeneration is possible and where artificial planting is a tool to manage problems with forest regeneration, e.g. competing vegetation etc. We consider it a high priority and responsibility to regenerate reasonably soon after harvesting. This is actually the basis for the national forestry law. Already Linneus had urged for regeneration of forest stands because "land left open is an unpleasant sight". To succeed in regenerating forest stands is of high importance in conserving forest genetic resources. More information is needed about the effective numbers of trees involved in natural regeneration. The level and distribution of relatedness is very different in a stand compared with a seed orchard with its totally unrelated clones.

The motivation for using artificial measures to regenerate forests is basically the search for control in time and space. Better growth can be achieved by facilitating the regeneration phase and by the use of preferred provenance. Planting is a good example of human action in a 'box', with many other natural processes ongoing, and to a large extent deciding, the final result. Examples from 10-year-old plantations in northern Sweden show that the regenerated areas consist of more naturally established seedlings than those planted originally. Planting as a method is a determinant, but very often the following management procedures have a decisive effect on changes in genetic diversity. If a maximum genetic diversity is aimed for then controlled artificial regeneration is the only possible way.

Plant production under perfect environmental conditions and with strict economic constraints, such as in greenhouses, avoids the selective processes that naturally take place. Diversity may be increased but in an unnatural direction and may lead to setbacks.

Gene conservation in the forestry practice

Gene conservation efforts are undertaken at different levels: species, alleles and population structure. Genetics is a science under rapid development.

Genetic resources are still characterized by indirect measures such as phenotypic expressions. It is a quite rough interpretation of genetic variability (not phenotypic variance). Gene activation processes and the mechanisms of promotors are largely unknown. These, along with the very recent molecular techniques, are to a large extent still to be developed and sound interpretation patterns need to be established.

By definition, science provides the best knowledge we have but never the final truth. Humbleness in the discussion of methods to be used in gene conservation is needed. We are talking about ecosystems which do not recognize national borders, therefore, international cooperation is indeed very important International cooperation also encourages the exchange of views, supports the development of strategies and promotes diversity, including the diversity found in different views and opinions. Let a number of flowers flower within regions as well as countries.

Forests in the former Soviet Union: investigating genetic resources of pine, spruce and fir species

Grigori G. Goncharenko, Vladimir E. Padutov, Arkadi E. Silin and Alexander E. Padutov
Institute of Forestry, National Academy of Sciences, 246654 Gomel, Belarus

Introduction

The majority of boreal forests in Eurasia are located in the territory of the former Soviet Union (eastern part of Europe and Siberia). The principle forest-forming components of these stands are pines, spruces and firs which are distributed in an area of 220 million ha from the Baltic Sea to the Pacific Ocean (Forest Encyclopaedia 1985). Regardless of the high variability and important economic and ecological role of the coniferous stands throughout eastern Europe and Siberia, detailed studies of their genetic resources have only commenced.

This is mainly owing to the lack of methods to carry out population genetic investigations. At present one of the most suitable methods for the study of genepools remains isoenzyme electrophoresis that enables the evaluation of genetic resources of populations or species. By the early 1990s, in different countries of the world, the genetic resources of more than 50 coniferous species had been studied by using isoenzyme electrophoresis (Yeh and El-Kassaby 1980; Conkle 1981; Guries and Ledig 1982; King *et al.* 1984; Gullberg *et al.* 1985; Ledig 1986; Schiller *et al.* 1986; Müller-Starck and Gregorius 1986; Cheliak *et al.* 1988; Millar *et al.* 1988; Moran *et al.* 1988; Paule *et al.* 1990; Kuittinen *et al.* 1991; Gömöry and Paule 1993; Boscherini *et al.* 1994; Hawley and DeHayes 1994; Edwards and Hamrick 1995).

The objective of our investigation was to provide an evaluation of the genetic resources of the pine, spruce and fir species distributed in the former USSR by using isoenzyme gene loci. The long-standing research in this area, conducted at the Laboratory of Molecular Genetics and Forest Tree Breeding at the Institute of Forestry (Gomel), was developed in accordance with the programmes of the former Committee for Forestry of the USSR and the Academy of Sciences of Belarus.

Genetic analyses

During the first stage of our research we worked out methods of electrophoretic analysis for 11 to 16 enzyme systems in the pine, spruce and fir species (Goncharenko *et al.* 1987, 1990, 1992a, 1992b, 1995a, 1995b; Goncharenko and Potenko 1991, 1992; Goncharenko and Padutov 1995). As a result of the genetic analyses it was confirmed that the enzyme systems assayed were encoded by 21-25 different genes. Acid phosphatase, alcohol dehydrogenase, aspartate aminotransferase, diaphorase, fluorescent esterase, glutamate dehydrogenase, glutamate-pyruvate-transaminase, glucose phosphate, isomerase, glucose-6-phosphate dehydrogenase, hexokinase, isocitrate dehydrogenase, leucine aminopeptidase, malate dehydrogenase, malic enzyme, phosphoenolpyruvate carboxylase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, shikimate dehydrogenase and sorbitol dehydrogenase were the enzyme systems assayed in electrophoresis on 13-14% starch gel (see Goncharenko *et al.* 1989, 1992b).

The number of trees sampled from populations ranged from 10 to 60 and averaged 26. Within each population trees were sampled randomly along transects, the minimum distance between individuals being greater than 50 m. Individual trees were genotyped using 8-20 megagametophytes plus embryos for every locus. The megagametophytes and embryos were sampled randomly from a set of no less than 50 seeds extracted from 2 to 30cones from each tree. Only enzymes with proved genetic determination and the mode of inheritance established were used for the analyses (Goncharenko *et al.* 1989, 1992a, 1993a, 1994; Goncharenko and Potenko 1991; Goncharenko and Padutov 1995).

Measures for evaluating genetic resources in natural populations of conifers

For a long time population studies of forest tree species have been based on their phenotypic traits. However, the performance of these traits often depends on environmental conditions, their coefficient of inheritance is low and, therefore, does not reflect the actual level of genetic variation of populations and species. The situation changed after isoenzyme electrophoresis became widely used in population genetic studies. In the 1970s population geneticists belonging mainly to Dobzhansky's scientific school (Lewontin and Hubby 1966; Prakash *et al.* 1969; Richmond 1972; Ayala and Powell 1972; Ayala *et al.* 1972; Lewontin 1974; Prakash 1977) developed a set of population genetic parameters. These parameters enable researchers not only to describe adequately the genetic structures of the populations investigated but also to estimate levels of genetic diversity and evaluate the status of genetic resources without any excessive expenditure of finance and time.

Among the basic genetic parameters used in our research are: percent polymorphic loci (P), mean number of alleles per locus (A), expected heterozygosity (H_e) and observed heterozygosity (H_o). Percent polymorphic loci (P) is calculated by dividing the number of polymorphic loci (having two or more various alleles) by the overall number of loci surveyed. This is normally calculated according to two criteria of reliability: at 99% (the frequency of the most common allele is not greater than 99% (P_{99}), or 95% (P_{95}). The mean number of alleles per locus (A) is calculated by dividing the number of the alleles revealed by the overall number of the loci surveyed. In some cases the mean number of non-rare alleles per locus ($A_{1\%}$) is used. To calculate this parameter only alleles with frequencies not greater than 1% (i.e. non-rare alleles) are used. Expected and observed heterozygosities estimate the level of genetic variation within populations most accurately. The observed heterozygosity (H_o) is calculated for a particular locus by dividing the number of hetorozygous trees by the overall number of the individuals surveyed. The expected heterozygosity (H_e), which depends on the population sample to a lesser extent than other parameters, is calculated for a particular locus using allelic frequencies as follows:

$$H_e = 1 - \sum x_i^2$$

where x_i is the frequency of the i-th allele.

Expected and observed heterozygosity means are calculated as mean arithmetic of it values for all the loci:

$$\overline{H} = \frac{1}{L} \sum H_J$$

where H_j is the heterozygosity of the j-th locus and L is the number of the loci surveyed.

The parameters used in our studies are based on allelic frequencies of 21–25 loci.

Genetic resources of pines

Seed material collected from approximately 1500 individual trees of ten pine species found in the territory of the former USSR was used. More than 60 natural populations from Latvia, Belarus, the Ukraine and Russia, including Siberia and the Far East, were assayed (Fig. 1 and 2).

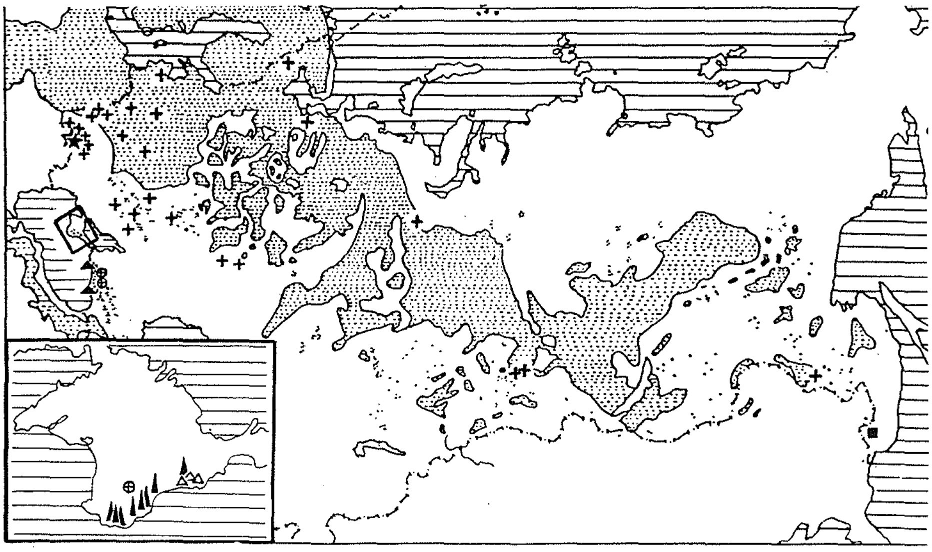

Fig. 1. Locations of 45 natural two-needle pine populations studied in the territory of the former USSR: + - *P. sylvestris* var. sylvestris; ⊕ - *P. sylvestris* var. hamata; ✱ - *P. mugo*; ▲ - *P. nigra*; Δ -*P. stankewiczii*; ▴ - *P pithyusa*; ■ - *P. funebris*. Natural distribution of Scots pine (▨) follows Pravdin (1964); Critchfield and Little (1966).

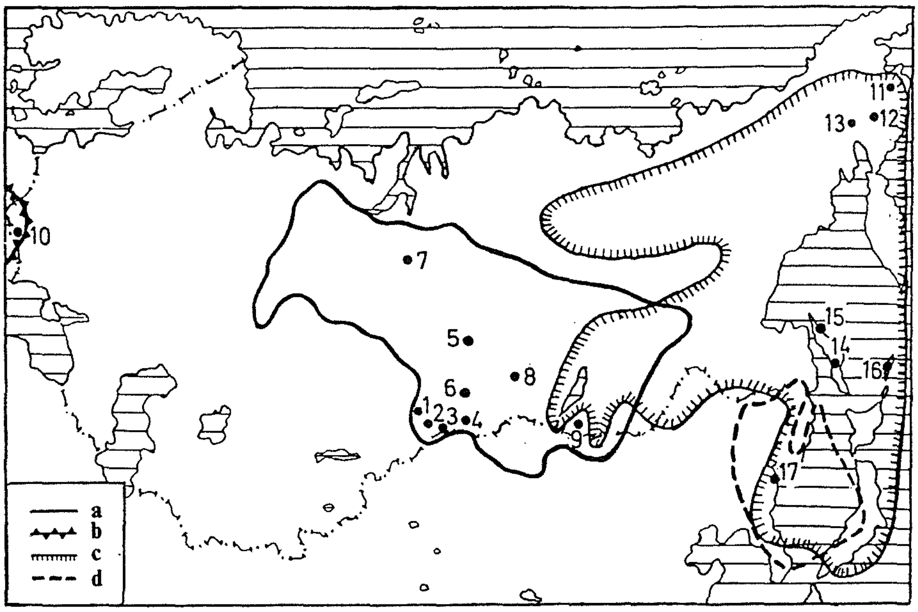

Fig. 2. Location of 17 natural stone pine populations studied in the territory of the former USSR: 1-9 *P. sibirica* (a); 10 *P. cembra* (b); 11-16 *P. pumila* (c); 17 *P. koraiensis* (d). Natural distribution of the stone pine species follows Critchfield and Little (1966).

Several species of the *Sylvestres* subsection - two-needle pines (*P. sylvestris* var. sylvestris, *P. sylvestris* var. hamata, *P. mugo, P. funebris* and *P. nigra*) exhibit high values of the genetic parameters used (Table 1). The percent polymorphic loci varies from 73-90% and the average heterozygosity is 25–28%. Among the five-needle pines (*Cembrae* subsection) high variability was obvious only in Japanese stone pine (*Pinus pumila*). *P. sylvestris* shows the highest variability among all the pine species studied, the mean number of alleles per locus being 4.5.

Table 1. Genetic variation in pine species

Species	P_{95}	P_{99}	A	H_e	H_o
P. sylvestris var. sylvestris	0.714	0.857	4.476	0.283	0.274
P. sylvestris var. hamata	0.76	0.905	3.000	0.265	0.264
P. mugo	0.652	0.826	2.609	0.267	0.254
P. funebris	0.739	0.739	2.043	0.263	0.309
P. nigra	0.609	0.783	3.000	0.259	0.252
P. pithyusa	0.304	0.348	1.522	0.091	0.086
P. stankewiczii	0.435	0.435	1.522	0.125	0.124
P. koraiensis	0.500	0.500	1.800	0.212	0.233
P. pumila	0.682	0.773	2.727	0.263	0.271
P. sibirica	0.450	0.500	1.900	0.174	0.171
P. cembra	0.400	0.400	1.450	0.116	0.158

Figure 3 illustrates a very wide distribution of heterozygosity values in pine species studied by several authors. The values range from 0% (*P. torreyana*) to 28.3% (*P. sylvestris*) the latter was observed in our investigations. Thus, according to the data presented, Scots pine is the most polymorphic species in the genus *Pinus* according to analyses at 20 and more loci. Interestingly, in the *P. sylvestris* populations studied, 13 unique (private) alleles were detected, that is, alleles which occur in only one particular population. Such alleles were revealed in both isolated populations and those from the continuous distribution area of Latvia, the Ukraine and Russia. The availability of a great number of private and rare alleles may indicate that the current micro-evolutionary processes are intensive. Presumably this explains the great plasticity of this species and its capacity to grow under extremely different conditions, from tundra and swamps to mountains. It should be noted that in several populations some of the private alleles have a frequency of 3–5%. The presence of the private alleles in local populations should be taken into account for gene conservation and reforestation programmes.

It should be emphasized that even in isolated *P. sylvestris* populations from eastern Europe a high level of genetic variation is preserved. These populations were not subject to inbreeding. Thus, each *P. sylvestris* population analyzed, including several isolated ones, possesses a high genepool and is a source of valuable genetic material.

The great variability observed in *P. sylvestris* indicates the high potential of this species for breeding. We compared natural stands, seed orchards and artificially planted stands of *P. sylvestris* located in the south of Belarus. It can be seen that the heterozygosity level in trees from seed orchards and artificial stands is lower than from within the natural stands. This result is expected and implies that the use of seeds from such seed orchards for reforestation may lead to reducing the genetic variation in future forests.

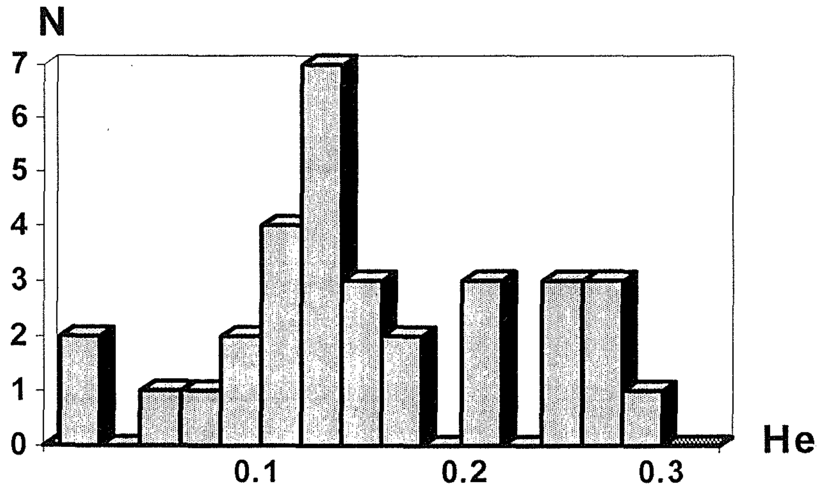

Fig. 3. Distribution of expected heterozygosity values for *Pinus* spp. N=number of species, H_e=expected heterozygosity. The data are taken from the current study as well as Goncharenko *et al.* 1993b, Gibson and Hamrick 1991; Mosseler *et al.* 1991; Boscherini *et al.* 1994; Goncharenko *et al.* 1994; Kim *et al.* 1994; Edwards and Hamrick 1995.

Genetic resources of spruces

The investigation was based on the seed material collected from more than 1000 individual trees of seven species occurring on the territory of the former USSR. A total number of 37 populations from Latvia, Belarus, the Ukraine, Kyrgyzstan and Russia, including the northern Caucasus, Siberia and the Far East, were assayed (Fig. 4).

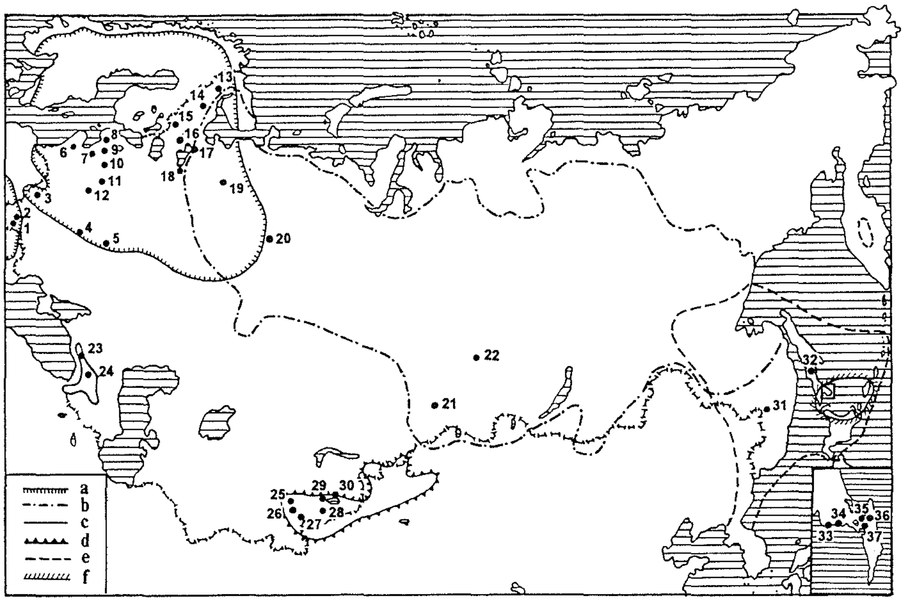

Fig. 4. Locations of 37 natural spruce populations studied in the territory of the former USSR: 1-12 *P. abies* (a); 13-20 *P. fennica*; 21-22 *P. obovata* (b); 23-24 *P. orientalis* (c); 26-30 *P. schrenkiana* (d); 31-34 *P. ajanensis* (e); 35-37 *P. glehnii* (f). Natural distribution of spruce species follows Shimaniuk (1974) and Pravdin (1975).

It is obvious that spruces belonging to the *abies-obovata* complex (*P. abies, P. fennica* and *P. obovata*), that has an immense distribution in Eurasia, exhibit the highest values of genetic variability (Table 2). Lower values are characteristic of mountain species such as *P. schrenkiana* and *P. orientalis* having small ranges in the Caucasus and Central Asia. The overall level of variability in mountain species is lower than in the species from lowlands.

Table 2. Genetic variation in spruce species

Species	P_{95}	P_{99}	A	H_e	H_o
P. abies	0.500	0.846	3.654	0.193	0.195
P. fennica	0.577	0.731	3.000	0.209	0.222
P. obovata	0.577	0.731	2.308	0.198	0.223
P. orientalis	0.417	0.625	2.000	0.159	0.159
P. schrenkiana	0.458	0.458	1.875	0.140	0.114
P. ajanensis	0.573	0.752	2.092	0.186	0.201
P. glehnii	0.520	0.582	1.732	0.185	0.195

When analyzing *Picea abies* occurring in the territory of Belarus, we estimated levels of genetic variation in both natural populations and seed orchards. Contrary to *Pinus sylvestris,* both types of stands showed similar levels of heterozygosity.

Genetic resources of firs

Seeds were collected from six fir species found in the territory of the former USSR. A total of 16 natural populations from Belarus, the Ukraine, Kyrgyzstan and Russia, including the Caucasus and the Far East, were assayed.

Abies sachalinensis revealed more than 80% of loci polymorphic and every tree was heterozygous for 20% of its genes. At the same time, the fir species studied showed lower levels of genetic variation than the pine and spruce species in general.

Table 3. Genetic variation in fir species

Species	P_{95}	P_{99}	A	H_e	H_o
A. alba	0.381	0.524	1.905	0.123	0.118
A. nordmanniana	0.429	0.619	2.476	0.111	0.106
A. sachalinensis	0.619	0.810	2.190	0.207	0.200
A. nephrolepis	0.143	0.143	1.143	0.071	0.143
A. sibirica	0.429	0.571	1.857	0.145	0.131
A. semenovii	0.048	0.095	1.143	0.015	0.017

It should be noted that among the fir species studied there is one in which the level of genetic variation is very low: *Abies semenovii.* It is distributed in an extremely limited area in the Tian Shan Mountains and is composed of a very small number of trees. According to our data, this species experienced the bottleneck effect and as a consequence its small number of individuals is under serious threat of extinction. This species was included in the Red Data Book of the former USSR. It is a good example of the association between the level of genetic variation and other parameters such as viability, demographic patterns etc.

Conclusions

On the basis of genetic parameters such as percent of polymorphic loci, mean number of alleles per locus, expected and observed heterozygosities, we could estimate levels of genetic diversity in pine, spruce and fir species found in the territory of the former USSR. It was shown that among the members of the genus *Pinus, P. sylvestris* has the widest distribution range and the highest level of genetic variation. On the other hand, for *P. cembra, P. pithyusa* and *P. stankewiczii* whose ranges are limited, the genetic variation at investigated loci is rather restricted. In the genus *Picea* the species of the *abies-obovata* complex show the highest levels of genetic variation. Low values are typical for mountain species (*Picea orientalis* and *P. schrenkiana*). In the genus *Abies*, the highest level of genetic variation is characteristic of *A. sachalinensis*. The lowest overall values were found in endemic *A. semenovii*. On the whole, the data obtained in our studies support the conclusions drawn by Nevo *et al.* (1984) and Hamrick *et al.* (1992). They inferred that species with a high level of genetic variation usually occupy a rather wide distribution range and possess ecological plasticity, while in a local species the values of genetic parameters are low. Thus, as a result of our research we obtained data on the status of genetic resources in conifers found in the territory of the former USSR.

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Forests in central Europe: economic transformation and its implications for the conservation of forest genetic resources

Karel Vancura
Forestry and Game Management Research Institute, Jiloviste-Strnady, 156 04 Praha 5, Czech Republic

Introduction

The Czech Republic is one of the countries where, because of the comparatively high population density and growing economic potential, forest ecosystems are severely threatened. The impact of air pollution and other intensive human activities is apparent in a significant proportion of forest stands. Vast regions are devastated, with only 40% of the forests without signs of damage by air pollution. Even the damaged forests represent important biological resources and contribute to the protection of natural environment. However, despite the recent forest decline associated with air pollution, since the beginning of the century there has been a tendency for the total forest area to increase.

Starting more than 200 years ago, efforts to intensify the production of timber led to the establishment of monocultures not suited to the given sites. This led to the reduction of both genetic diversity and quality of the stands. As a result, Norway spruce (*Picea abies*) is now the dominant species covering 55% of the total forest area. Commercial forests occupy nearly 96% of the forest area (1 489 300 ha).

A structural approach in forestry is very important at the present time because communal and private forest holdings have only just been re-established within former boundaries, but their management is being undertaken in a completely new and different social, technical and economical framework. The underestimation of the consequences of the transformation process could, beyond the forestry sector itself, have broad socioeconomic implications.

Status and conservation of forest genetic resources

The significant changes that occurred in the species composition of stands as a result of intensive forest management in the past are probably the main reason for the frequent damage, and sometimes even disasters, in our forests. Forest soil has been modified to a significant extent as the result of human activities. Reforestation practices since the second half of the 18th century were the beginning of the significant changes in the species composition because of the planting of mainly coniferous species. It means that remnants of natural populations of main forest tree species have been preserved only in some localities, often as over mature stands. Their value as a potential source of reproductive material is considered in the government programme of environmental protection (1985).

The genetic resources of main economic conifers (Norway spruce, Scots pine and European larch) are depleted in many regions, industrial pollution being the primary cause. The negative impact of drought, abiotic factors, insects and parasitic fungi is also important. Some improper silvicultural measures and heavy deer populations further multiply the forest damage.

The genetic resources of silver fir, elms, regional Norway spruce populations, and in some regions oak, beech, maple and small-leafed lime are endangered. Considering the objective of preserving the broadest genetic diversity, not only the most representative populations or important single trees are subject to gene conservation. Populations from marginal localities also need to be conserved, despite their lower economic importance as such populations and single trees may carry genes of importance for breeding (adaptability, resistance). *In situ* and *ex situ* measures are necessary to complement each other. High priority is given to gene conservation in the areas most affected by air pollution in the northern part of the country.

Nature protected areas are of great importance for maintaining or restoring natural or semi-natural, stable forest communities. Improving collaboration between nature protection agencies and representatives of the forestry practice in the Czech Republic have resulted in, among other things, the refusal of some extreme demands calling for the ban of any intervention in the nature reserves. Many valuable elements of the protected areas could hardly be preserved without minimal management interference. Seed collecting is more common in stands under a special management regime known as 'gene bases' (see below).

The fundamental task of the regeneration of forests in heavily damaged, mainly mountainous regions is, besides lowering the pollution load, to safeguard most endangered genetic resources of natural forest tree populations. After logging of confers, deforested areas can be reforested only with difficulty. In the framework of the programme on environmental protection, there are different projects, prepared in many regions, with the aim of gradually restoring semi-natural forest communities with the corresponding species composition, origin and spatial structure (stability). The fact that in many cases the position of nature protection authorities and forestry institutions have come closer in recent years, is considered as a very positive approach yielding concrete local agreements and cooperation.

During the latest period, an increased effort to conserve and enhance the genetic resources of forests has been undertaken on the basis of present knowledge about variability and heritability. Provenance research is important from the viewpoint of the choice of suitable provenances and their transfer. Studies of intra-specific diversity should also be viewed as an instrument of saving the genepool. Very rare populations, for instance, can often be found in provenance or progeny field trials. Genetic marker studies have been initiated.

Results of provenance research provided information on the geographical variability of qualitative and quantitative traits, and reactions of various populations to different site conditions. These results are applied in forestry practice through rules for the use of different provenances, delimitation of seed zones and recommendations on the transfer of reproductive material.

If natural regeneration as a preferable method cannot be used, than reproductive material is obtained from forest stands and individual trees approved for collecting. Its transfer is only possible in accordance with specific guidelines (i.e. mainly within seed zones delimited for main economic species). The documentation is considered very important; when marketing and transferring seeds, it is obligatory to give the source of the reproductive material as part of the certificate of origin or accompanying documents for distribution. However, the basic principle for seed legislation is the use of approved reproductive material of local origin.

Institutional framework

The national concept of forest gene conservation is based on research recommendations and the long-term experience provided by institutions administrating state forests. Considering the current economic restrictions, the concept is minimal, nevertheless it is the basis which should be used in legislation focused on the conservation and enhancement of genetic resources.

In considering the use of forest genetic resources, there is the specific role of the Forestry and Game Management Research Institute (FGMRI). The department for certification and inventory of reproductive sources ensures the identification, designation, inventory and control of sources of forest reproductive material. Its consulting activity is also of importance, mainly for new forest owners.

Prominent goals of the national forestry policy include improving biological diversity and approaching the natural species composition in forests. This is considered necessary for improving the overall stability of the forest stands.

The natural, actual and target species composition in the forests of the Czech Republic is as follows:

Species composition (in %)	Natural	Actual	Target
Norway spruce	11.2	54.3	40
Silver fir	19.8	1.0	2
Scots pine	3.4	17.6	11
European larch	0	3.4	15
Dwarf pine		0.3	
Douglas fir and other introduced		0.7	
Other conifers		0.3	
Total conifers	**34.7**	**77.2**	**68**
Oak	19.3	6.1	9
Beech	40.1	5.5	11
Hornbeam	1.5	1.2	
Maple	0.7	0.7	
Ash	0.6	0.9	
Birch	0.8	2.9	
Alder	0.6	1.4	
Lime	0.8	0.9	
Poplars		0.5	
Willows		0.1	
Black locust		0.5	
Other broadleaves	0.2	0.3	12
Total broadleaves	**65.3**	**21.2**	**32**

Economic transformation in forestry

In 1937 the proportion of private forests amounted to 1 370 000 ha (58% of total forest land), large and medium size estates prevailed, but 30% of private forest properties had an area of less than 10 ha.

As the result of political developments after 1945, 810 000 ha of private forests became state property and by revision of the first land reform (1947) a further 348 000 ha were added. Communal forest properties were also nationalized (450 000 ha). In the 1950s state ownership reached 70% and by 1989 it was 97% of the total forest land (the remaining 3% were cooperative farm tenures).

As part of the re-privatization process during the early 1990s, more than 145 000 applications of former owners for the restitution of their property rights were registered (total area an estimated 588 000 ha). Agreements on the restitution of forests were authorized for 70.7% of the area, mainly properties of more than 200 ha for which the documentation was more complete. Approximately 2900 municipalities asked for the return of 248 000 ha of forest, most of which has been handed back.

The economic reform of forestry in the Czech Republic does not consist only in the restitution of the previous ownership rights, the following steps have also been taken:

- Creation of the legal entity, Forests of the Czech Republic (LCR). A significant part of the former State Forest property is owned by this new association established by the government in 1992. It has 26 Regional Forest Districts and 96 Forest Administrations.

- Transfer of the management rights in state-owned forests from seven state enterprises to the LCR.
- Privatization of state enterprises through 94 joint-stock companies and about 30 other entities.
- Possible privatization of a part of the remaining state forest property is anticipated.

This process affected the overall state of forests mainly with regard to forest protection and consequently genetic resources. The main problem is that the respective valid directives and guidelines were prepared at the time of exclusive state forest ownership. Particularly small forest owners and traders may not be able to comply with the above principles and guidelines of forest management.

Besides natural regeneration, the following measures aimed at the conservation and reproduction of genetic diversity in forestry practice have been implemented on state and other (private and communal) property (as of December 1995).

Unit	Total ha	State ha	State %	Other %
Gene bases	124 863.51	96 671	77.4	22.6
existing	66 889.36	52 194	78.0	22.0
proposed	57 974.00	44 477	76.7	23.3
Approved seed stands	145 298.19	99 443	65.0	35.0
Conservation stands	3 808.85	2 467	64.8	35.2
Seed orchards	326.71	300	91.3	8.7

Stands approved for seed collecting: A positive trend of increasing the proportion of broadleaves has been continued during recent years. The central inventory (database) is regularly updated. It is foreseen that only approved stands belonging to one owner be included in forest management plans which may exclude small-scale forest owners from the scheme. In close cooperation with forest owners, ÚHÚL (Forest Management Planning Institute) and the forest service made a new proposal for phenotypic classification of stands.

Gene bases: In addition to the currently existing 275 gene bases, another 135 were agreed upon, the rest is in the proposal stage and will be discussed gradually during the preparation of new forest management plans. The problems with gene bases on newly acquired private forest land are being negotiated.

Seed orchards: The total area is 326.7 ha (conifers 301.05 ha and broadleaves 25.66 ha). The distribution according to ownership is shown in Figure 1.

Conservation stands (seed stands): These are established from seeds collected in the approved stands with best phenotypic performance. There is a total area of 3 808.85 ha, of which 75% were planted and 25% resulted from natural regeneration of certain approved stands. The legal implications of establishing conservation stands on private property are under discussion.

Selected trees: The total of selected trees is 7727, conifers 6495, broadleaves 1232. There is no accurate information about their distribution in the private stands (e.g. about 19% of selected trees in northern Bohemia are on private property).

Seed and genebank: A seed centre in Tyniste nad Orlici is owned by the state forest association LCR. Originally aimed at supplying genetic material for breeding programmes, the seed bank also serves for gene conservation purposes, especially for regions most affected by air pollution. Its activities cover the entire territory of the country and so far have focused on the long-term storage of main commercial species.

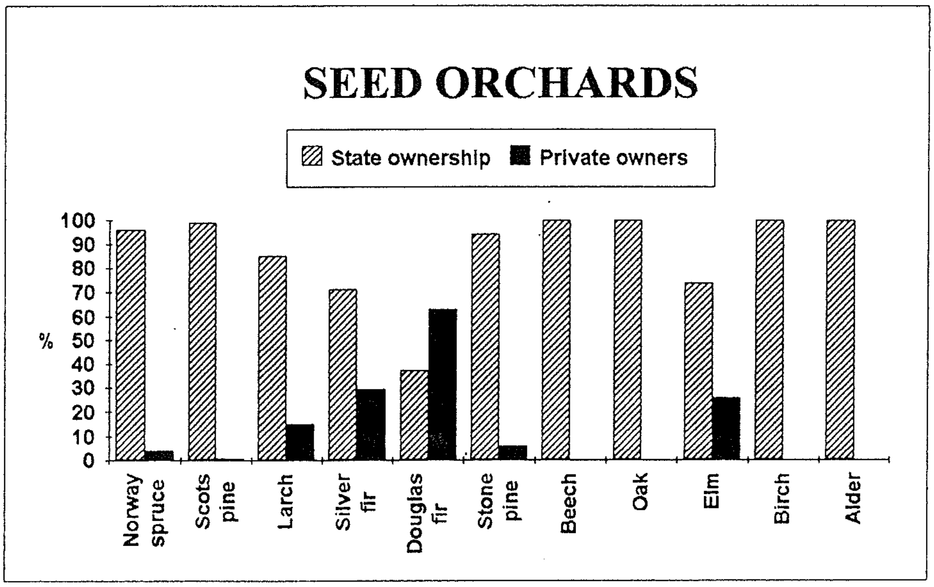

Fig. 1. Distribution of seed orchards, according to ownership.

The mentioned units in which active measures aimed at the conservation and reproduction of genetic diversity are taken represent more than 274 000 ha of forests, i.e. an estimated 10.4% of the total forest area. Their further development will depend on the process of privatization. It is assumed that approximately one half of the total forest cover will remain in state ownership.

Present experience shows that approximately 10% of the new owners refuse to accept the conditions necessary for the designation and sound management of gene bases. The area of one gene base usually covers groups of several forest stands, i.e. estates of various owners and when one of them disagrees, it is often not possible to continue in the procedure. There is an enormous potential for education and improving public awareness, as well as a completely new legal framework.

Genetic sources and related matters are mentioned in several paragraphs and articles of the new Forest Act. Forest owners, for example, are obliged to maintain and conserve the genepool of forest tree species by ensuring the following measures during reforestation:

- Seeds, seedlings or transplants from the same natural forest region, elevation (forest vegetation zone) should be used. Norway spruce, Scots pine and larch have to be collected from selected trees, approved stands or seed orchards. The regulations are to be expanded to include further species.
- Approved stands, selected trees and other units are protected by a prolonged rotation prescribed in the forest management plans.
- The Ministry of Agriculture nominates an institution responsible for documenting and monitoring all related items (*Forestry Research Institute*).
- The import of seeds requires the consent of the Ministry on the recommendation of the responsible technical institution.
- Collecting, processing and marketing of forest reproductive material will be licensed.

Conclusions

Responsible, prudent forest management, fulfilling the multiple forest functions, is unimaginable without the appropriate concern about the genetic resources which form its basis.

The Czech Republic, a signatory state of resolutions from Ministerial Conferences on the Protection of Forests in Europe, is committed to the conservation of the genepools of forest tree species and to maintaining biodiversity in general. In addition, the intention of eventually joining the European Union necessitates the implementation of a policy of international collaboration with regard to reproductive material.

It is necessary to ensure appropriate control of trade in forest reproductive material because at present traders are only obliged to register in the general commerce register. The new Forest Act adopted by the Parliament of the Czech Republic is a basis for developing legislation in this field. Responsibility of individual foresters, forest owners and state forest officers is, of course, of high importance.

Conservation of forest genetic resources in the Mediterranean region

Riccardo Morandini
Istituto Sperimentale per la Selvicoltura, 52100 Arezzo, Italy

Introduction

More than 80 years ago, Robert Hickel, a French forester long active in Algeria, called upon his Mediterranean colleagues to join forces to develop specific guidelines and management methods to ensure the conservation and improvement of Mediterranean forests. He stressed that "it would be a mistake to apply methods and ideas coming from the countries of fog in the Mediterranean region, a country of light".

Mediterranean environment

Light is, in effect, a basic characteristic of the Mediterranean environment, where the sun normally shines 300 days of the year. Sun means heat and high evapotranspiration in a region where rainfall is often scarce and predominately seasonal, leading to long periods of intensive drought, often aggravated by strong dry winds.

Rainfall does not normally amount to more than 500 mm, is seldom over 1 000 mm and often is only 200 mm. This amount fluctuates extensively from year to year. Rainfall is strongly concentrated during the autumn and winter, whilst only a small fraction, if any, falls during the summer. The dry period can last for as long as four to six months and even longer in the south. Temperatures also show strong variations, and frost, although generally light, is not unusual. Maximum temperatures often exceed 40°C and can be as much as 60 or 70°C in the soil.

The latitudinal extension of the region, from 30° to 45° north, leads of course to ample variability, further complicated by a marked topography. All around the Mediterranean, the mountains influence local ecological conditions and soil is often heavily eroded on the steep slopes.

Down through the ages, under these harsh conditions, a particular type of forest flora developed having specific characters of adaptation, plasticity and resistance to an adverse environment, especially to aridity. The forest flora is characterized by a strong resilience often linked to a high tendency towards agamic reproduction.

Human impact and collaboration

In addition to adverse ecological conditions, Mediterranean forest trees and shrubs have been submitted to intensive human activity for several millennia. The extent structure and composition of the forests have been deeply modified through human action such as timber harvesting, and the search for more agriculture land and grazing. Recently, the main negative influences have been associated with urbanization and tourism.

Over the last few decades, socioeconomic development and population growth have led to the evolution of a different type of human impact on the forests. While on the northern shores of the Mediterranean, despite forest fires, forest cover is increasing, in the south they are being destroyed at an alarming rate.

According to an FAO study, in the southern and eastern regions of the Mediterranean, deforestation has reached 1% per annum, while the rate of deforestation for the whole of Africa is estimated at 8%. Although the northern shore situation is not so dramatic, there are also problems of forest conservation, which concern provenances rather than a species.

As a result of its common concerns, unity, long established links and cooperation it is difficult, if not impossible, to consider only a part of the Mediterranean region. Following Hickel's request, a league of Mediterranean foresters was established in 1922. Until 1938 the league actively dealt with several problems of forest conservation and restoration. In 1948, FAO created an inter-regional Sub-Commission on Mediterranean Forest Problems, a statutory body which continued and strengthened the activity of the old league under the name of Silva Mediterranea. Within this framework, a special Committee on Mediterranean Forest Research Problems was established in 1962. This committee worked in close cooperation with the IUFRO and with several research networks on both sides of the Mediterranean.

Some of the main themes addressed in a series of active networks were the selection of Mediterranean conifers; the establishment of arboreta; the selection of some exotics, namely *Eucalyptus*; and the protection of alfa (*Stipa*) formations. This cooperation continues, namely on the selection of conifers, especially *Pinus pinea* and *Cedrus*, and on multipurpose trees and shrubs. These activities are carried out in connection with IUFRO, the United Nations Educational, Scientific and Cultural Organization (UNESCO) and its Man and Biosphere Programme (MAB), and with the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM).

Status of forest resources

It has been estimated that 25 000 species are represented by Mediterranean forest flora, several of which are strictly endemic. Most species come from Europe, Asia and Africa, as gradual transitions and often hybridization occurred with other species. For example, the beech tree, *Fagus sylvatica*, occurs across the Alps and along the Italian peninsula to Sicily, as well as in northern Europe, whilst in the southern Balkans and Turkey it meets *Fagus orientalis*.

The group of Mediterranean firs is even more complicated, where, besides *Abies alba*, we find *A. cephalonica*, *A. nordmanniana*, *A. cilicica*, *A. numidica*, *A. pinsapo* and a large series of transition forms. Despite research already undertaken, much work still needs to be done on taxonomy, i.e. on the exploration, selection and sampling of *A. nordmanniana* and other Turkish firs.

Several conifers are found around the Mediterranean. There are various species of the genus *Abies*, but also *Cedrus*, *Cupressus*, *Juniperus*, (*J. communis*, *phoenicea*, *thuriphera*, *excelsa*); *Pinus* create extensive pure stands or mixed stands with broadleaved trees, depending on site conditions, vegetation evolution and human intervention. Many pure pine stands of both *P. halepensis* and *P. pinaster* have grown up after forest fires owing to their aggressive form of seed dispersal. *Pinus brutia*, another species of Mediterranean interest, has been investigated in the extended group of *P. halepensis*.

Among oaks, the well known cork oak, *Quercus suber*, is characteristic of the western Mediterranean region including west Tunisia and Morocco. The species is also present in western Turkey. On the other hand, the typical Mediterranean oak is *Quercus ilex*, holm oak, which is the most important and evolved element in large tracts of the Mediterranean macchia. The macchia is an extremely rich forest formation created by several tens of minor species, such as *Laurus nobilis*, *Arbutus*, *Phillirea* and also, under better site conditions, by *Fraxinus oxyphilla*, *Sorbus aria*, *S. torminalis*.

At least 15 species of other oaks are present at higher altitudes. These are represented by *Q. pyrenaica* to *Q. conferta*, *Q. afares*, *Q. libani*, *Q. pubescens*, *Q. cerris*, *Q. petraea* and other semi-deciduous or deciduous species.

Among other broadleaved genera, *Acer*, *Alnus*, *Castanea* are present and important locally, whilst in the southern and eastern regions several *Acacias* are dominant and often essential to local forestry. In many Mediterranean areas several broadleaved species are important, not only for timber production but also as a source of fodder, whilst several species supply other edible products, essential oils, gums, etc.

These brief points delineate the richness and complexity of Mediterranean forest flora, which despite difficult ecological conditions and profound human influence, still covers large tracts of the region.

The bases for this strong resilience are abundant seed production and/or a strong tendency towards resprouting, both from stump and roots. This ensures the gradual re-constitution of forests when negative factors disappear. In effect, strict protection measures, like fencing, often ensure the progressive evolution of the forest in a relatively short time.

Under the natural and evolving socioeconomic conditions of the various regions, any action aiming at the protection, conservation and improvement of the forest cover and its genetic resources meets with occasional and specific obstacles.

Contrasting problems, all being factors of forest disturbance, are presented by human behaviour with its excessive demand for timber and grazing in the south; or the intensive and concentrated presence of tourists, and the total abandonment of large forest areas in the north. The main concerns are: a more rational use of land and the prevention of forest fires. Both these goals should be given primary attention in the framework of the Mediterranean Forest Action Programme recently launched by FAO in the context of the Silva Mediterranea.

Activities towards conservation and sustainable use of forest genetic resources

Several programmes are under development or are currently in progress, to tackle specific problems, both *in situ* and *ex situ*, on the conservation of Mediterranean forest genetic resources.

Exploration and evaluation activities for several Mediterranean tree species started in the 1920s under the old Silva Mediterranea. The first provenance trials on *Abies alba* began in 1929. Because of its clearly superior vigour, and more recently to its supposed higher resistance to air pollution, this led to the transfer of Calabrian fir provenance to Denmark and later to other countries in central Europe.

After 1948, a series of technical and ecological studies on *Cupressus* and *Cedrus* were developed under the auspices of FAO. One result of these ecological studies was the creation of a map, around 1955, of the Mediterranean region. This map was later improved in cooperation with UNESCO.

Better organized, more systematic and intensive programmes were developed in close cooperation with all Mediterranean countries of the south, east and north. During the 1960s, programmes commenced involving Mediterranean firs with the collection of 55 provenances of different pines from *P. halepensis–brutia*, which are now under trial in 11 countries, and the systematic selection of seed stands of *P. nigra, P. pinaster, P. pinea, Cupressus* including *C. lusitanica*, and the extremely rare *C. dupreziana*, which, like *Abies nebrodensis*, is on the verge of extinction. Gene reserves were established for both these indigenous and exotic species.

With the establishment of the FAO Panel of Experts on Forest Gene Resources, specific recommendations have been given and updated for action in the Mediterranean region.

The UNESCO-MAB programme provided an important incentive towards forest resources conservation. This led to the establishment of a series of biosphere reserves, several of which concentrated, or included, important forest tracts. This programme attracted the attention of the authorities of many countries and led to the creation of several national parks, or special protected areas, as in Turkey for example.

It should be mentioned that the development of the Organization for Economic Cooperation and Development (OECD) and European Union systems for the control of forest reproductive material provided a strong impetus for the selection of seed stands and, in general, for the reinforcement of programmes on forest genetic resources, not only in the Mediterranean.

One very successful network of Silva Mediterranea is coordinated by O. M'Hirit in Morocco and is active also in the Lebanon. This network explores and collects in the extensive *Cedrus* stands of Taurus in Turkey, in Cyrpus the strictly endemic *C. brevifolia* and *Cedrus* stands in the Atlas Mountains as well as in the artificial stands in France and Italy.

Other networks are occupied with *Pinus pinea* as well as other multipurpose tree and shrub species, with particular emphasis on the southern and eastern Mediterranean.

In situ conservation of forest genetic resources has, for several years, been attracting more attention throughout almost all the countries of the Mediterranean. Extensive forest areas are now placed under various regulations aimed at the conservation of natural resources. The main protected areas include: national or regional parks, nature reserves, biosphere reserves and biogenetic reserves. The degree of protection depends upon the scope and the objectives of the programme. Access can be restricted to scientific purposes (reserves), or limited to pedestrians. Depending on the scope of protection, local conditions, country regulations, management and harvest can be completely excluded or subject to special rules or limitations. Unique examples of *in situ* conservation areas are forest stands set aside for seed collection.

Conservation of valuable genetic resources *ex situ* is ensured in various ways. Arboreta and botanical gardens, some established centuries ago, often contain extremely rare tree species.

Seed orchards, species trials (both of indigenous and exotic tree species) and provenance trials, which are present in almost all countries, are rich and precious gene reserves. Among clone collections and clonal banks *Populetum Mediterraneum* deserves special attention. This is a rich collection of more than 200 poplar clones established close to Rome under the sponsorship of the International Poplar Commission of FAO. Another example is the chestnut, *Castanea sativa* collection at Vallombrosa in Italy.

Many forest tree species, especially conifers, can be conserved as seed for quite long periods, even decades. The establishment of a Mediterranean forest seed bank has been recommended and deserves more attention. For other species, modern advanced techniques enable the conservation of genetic material *in vitro*.

Outlook

Special mention should be made of programmes aimed at the conservation of Mediterranean forest tree species in danger of extinction as a result of human action, or natural pests and diseases. For instance, a stand of less than 100 trees of *Cupressus dupreziana* at the fringe of the Sahara should be under strict protection. Young trees of this species are grown in Algerian nurseries. In Morocco, attention is now placed on the conservation of the remnants of *Cupressus sempervirens* that were heavily attacked by *Seridium cardinale*.

The 29 remaining living trees of *Abies nebrodensis* in Sicily are under strict protection. Several hundred young trees have been successfully planted from seed in their natural habitat, and two grafted collections have been established in central Italy. *Tetraclinis articulata, Prosopis cinerea,* species of the genera *Ulmus, Castanea* and a few other species also require urgent attention.

The need is felt for more effective coordination, which is not yet fully ensured by the existing regulations or agreements. Within the framework of the newly established EUFORGEN, the establishment of national conservation plans could make a strong contribution towards this goal.

On a wider scale, the effective action of the FAO Panel of Experts on Forest Gene Resources could be further enhanced. The highlights and recommendations of the Panel, which acts also as the main consultant to IPGRI, serves as a solid reference base deserving our full attention.

Appendix

Recommendations of the European Forest Genetic Resources Workshop, 21 November, Sopron, Hungary

A. International Technical Conference on Plant Genetic Resources

Recognizing the importance of the International Technical Conference and its impact on the forestry in Europe, **noting** that in most countries, the overall management and the institutional structure responsible for forest genetic resources fall under a different administrative competence than the crops domain, and **emphasizing** that involvement of both crop and forest genetic resources experts in the Conference would be of mutual benefit in elaborating overall strategies, the Workshop **recommended** that:

- national delegations to the Conference include expertise on forest genetic resources;
- specific mention of forest genetic resources be included in invitation letters and background materials submitted to countries in Europe, and that correspondence related to the International Technical Conference be copied to Heads of Forest Services; and
- a circular letter drawing attention to this need, be prepared and distributed through the coordinating secretariat of EUFORGEN, in consultation with FAO.

B. Global Plan of Action

It was **recognized** that national strategies for the conservation of forest genetic resources should be elaborated in accordance with perceived national needs, institutional possibilities, and financial and resource related realities. These strategies should be established within a regional framework to ensure complementarity and overall coverage.

Strongly **supporting** the recommendations given by the preparatory workshops on forest genetic resources in, respectively, Berkeley, USA and Toronto, Canada, and in line with their recommendations, the European Forest Genetic Resources Workshop **recommended** that:

- each country establish and develop a national strategy for the conservation of forest genetic resources, and
- national strategies include a core set of common, key elements (such as lists of target species, methods to be applied and organizations involved).

The Workshop noted that a number of European countries had already developed formal national programmes on forest genetic resources. Through regional collaborative activities, countries which had not yet developed a national programme could benefit from operational programmes in other countries.

The Workshop **recognized** that EUFORGEN is a framework which can facilitate collaboration in the field of forest genetic resources in Europe. **Supporting** the recommendation of the European Regional Meeting on Plant Genetic Resources (Nitra, Slovakia in September 1995) and **considering** the outcome of the Steering Committee of EUFORGEN (Sopron, Hungary in November 1995), the Workshop **recommended**:

- that EUFORGEN be considered as the platform for the implementation of the forest genetic resources components of the Global Plan of Action in Europe and that this task be carried out in close collaboration with FAO, IUFRO and other relevant organizations.

Recognizing the basic link between conservation, tree improvement and managed use of forests, the Workshop **recommended** that:

- a balance be sought between *in situ* conservation of forest genetic resources and sustainable forest management on the one hand, and management of protected areas on the other, and
- conservation and management of genetic resources *in situ* and *ex situ*, and tree improvement activities be considered as complementary strategies.

Recognizing that there has been a long tradition of forest management and silvicultural practices in European countries, the Workshop **recommended** that:

- the activities on forest genetic resources and their conservation be increasingly integrated into applied silviculture and forest management systems.

Recognizing that protected areas potentially contribute towards the conservation of genetic resources within the framework of an overall conservation strategy for given target species, the Workshop **recommended** that:

- rules and legislation governing the collection of reproductive material (for conservation and research purposes) from strictly protected areas be carefully reviewed, in collaboration with competent authorities, to maximize benefits for all concerned.

Recognizing that lack of knowledge is a major constraint to effective genetic conservation, and fully **supporting** the recommendations made in this sense by the preparatory workshops on forest genetic resources in North America as well as by the European Regional Meeting on Plant Genetic Resources, the Workshop **recommended** that:

- steps be taken to coordinate research and harmonize research methods on a regional level;
- monitoring of genetic diversity of forest reproductive material be given high priority in research;
- research be conducted to improve silvicultural and forest management methods, in order to secure long-term maintenance and enhancement of genetic diversity;
- education be promoted and training opportunities be increased in the field of forest genetic resources, and
- public awareness about forest genetic resources be promoted at all levels.