The European forest genetic resource programme. Objectives and general concept. A case study concerning the black poplar (*Populus nigra* L.)

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Abstract

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The paper surveys the needs for protection of forest trees native to Europe, and the recommendations made and the actions taken by the European countries both in regard to *in situ* and *ex situ* conservation of genetic resources of forest trees. *Populus nigra* L. is presented as a model and a test case for orientation.

Introduction

Compared to cultivated plants, the domestication process acts on forest species for only very few generations. If some of them like poplars are beginning to be improved, most remain still wild.

Forest trees are long-living woody plants, allogamous, most of them diploïds and monoecious (most of the conifers), others dioecious (such as poplars). They are genetically more variable than any other living organisms (Hamkrick 1990). Nevertheless in Europe, numerous human activities could soon endanger this genetic diversity.

In fact, only few species of forest trees are now threatened at the species level (for example *Ulmus* sp. or *Abies nebrodensis*) but most of them suffer genetic erosion at the intraspecific level (ecotype, race).

Several authors recommended some time ago the conservation of forest gene resources (Bouvarel 1970, Schoenike 1975, FAO 1975, Kemp 1977, Namkoong 1989) but no European forest gene conservation programme exists until now.

The Ministerial Conference for the Protection of Forests in Europe (Strasbourg 1990)

The first ministerial Conference for the protection of forests in Europe was held in Strasbourg in 1990. Thirty two countries attended this Conference and signed six resolutions. One of these resolutions, the Resolution 2, was especially devoted to the conservation of the European forest gene resources.

Written in general terms, this Resolution 2 asked each State to develop a forest gene conservation policy and to apply it urgently through simple, efficient and long lasting measures; it also emphazised the need for a permanent tool of international cooperation (Proceedings of the Ministerial Conference, Strasbourg 1990).

We ought to underline the originality of the approach, planning from the beginning an international follow-up committee for each resolution, responsible for the translation of the resolution into an operational programme, its practical implementation, and the search for a permanent operation through the existing international organizations.

Resolution 2 focusses on *in situ* conservation, supplemented (or replaced) by *ex situ* conservation when relevant. Even if we have not all the genetic informationdesirable to organize the optimal conservation strategy for a given species, we must use all the available information to plan and to apply without any delay a gene resource conservation programme. To be realistic, Resolution 2 of the Strasbourg Conference recommended working species by species, beginning with the threatened and the most important ones, in terms of ecological and economic considerations.

Objectives and methods of conservation

For each species, the prime objective is to maintain or to increase the genetic adaptability. Such a strategy will allow adaptation to unpredictable changes of the environment and will offer possible adjustements to changes in human needs. For species of current economic interest for wood production, it will insure a continuous genetic improvement over several generations of recurrent selection.

Reaching this objective requires us to be able to identify and to maintain a representative sample of the genetic diversity in the species of concern. For the economically important forest trees of main economic interest (Norway spruce, Scotch pine, beech, poplars), we already have some old multisite provenance and progeny experiments as well data on the trends of their genetic structure, their adaptive traits and more recently, on molecular polymorphisms. Unfortunately, this situation is not general. For many of the species ecological and historical features, the breeding system and spatial distribution are the main guides for organizing genetic conservation.

In its recommandations, Resolution 2 of the Strasbourg Conference favoured *in situ* conservation, whenever possible.

For several forest tree species, represented by largely extended stands, an empirical approach has been used, in Finland and France for example (Koski 1991, Arbez 1992), to recommend gene reserve units of around 100 ha (10 ha for the gene reserve itself, and 90 ha for the isolation zone). This means 800 seed-bearers for natural regeneration of the gene reserve itself and tenfold more for the conservation unit as a whole. Such a large area also allows a given population to be represented by several age-classes, from young seedlings to mature trees, with an expected evolution of the gene diversity according to interannual variations in flowering, natural regeneration and selective pressure factors.

The main constraint for the sylvicultural management of these gene reserves is natural regeneration in the reserve unit and its isolation zone, replaced or completed by the planting of seedlings originating from local origin when necessary. In the present state of the genetic knowledge, commercial thinnings are still permitted. A pragmatic evaluation of the acreage necessary for such *in situ* gene conservation of beech and fir in France represents less than 1 % of the total acreage of the forest for each species.

The same figures were obtained in Finland for Norway spruce. Such studies show that *in situ* conservation is economically feasible, at least in public forests.

Ex situ conservation appears necessary in several cases:

- When harsh modification of the environment becomes incompatible with survival or natural regeneration of the species (acid rain in Central Europe for example, repeated forest fire).

- When urban extension and rural management procedures impose the suppression or a severe limitation on the size of the original tree population.

- When the general evolution of the forest management methods or the sylvicultural rules are clearly unfavourable for a given species.

- If a scattered spatial distribution or the mating system requiresan excessive area for the *in situ* conservation unit (noble hardwoods for example).

Traditional *ex situ* conservation plots are composed of seedlings originating from controlled seed collections made in the original populations, or from vegetative copies (grafts, cuttings) of individual trees. Taking into account the long life span of most forest trees, the problem of the modification of the genetic structure of the population according to the new adaptive pressures, or dealing with the sexual reproduction of the conservation unit over generations, have not yet been the subject of experiments.

Some methods, like multilines and multiple populations breeding system (Namkoong & al. 1980, Burdon & Namkoong 1983, Eriksson & al. 1993), theoretically offer a good solution for a dynamic management of the gene diversity of *ex situ* conserved forest trees, when performed on a multi-site scale.

Four pilot forest gene conservation networks

The Follow Up Committee of Resolution 2 proposed to begin a step by step conservation programme with four pilot networks (Report on the Follow Up of the Strasbourg Resolutions, Helsinki 1993). The species were chosen in agreement with the national delegates of the Strasbourg Conference. They were requested to answer to a questionnaire prepared in cooperation with FAO, IPGRI and CEU about the level of threats to forest tree species and populations in their country and their priorities for a

European forest gene conservation programme. Four different case studies were finally chosen to represent different models of spatial distribution (largely extended stands, scattered individuals, linear repartition) and different breeding and seed dispersal systems (wind and insect pollinated species, light or heavy seed species, wind or animal seed disseminated species).

The four pilot forest gene conservation networks deal with Norway spruce (*Pices abies*) cork oak (*Quercus suber*) noble hardwoods and black poplar (*Populus nigra).*

Norway spruce *(Picea abies)*

This conifer species has a wide distribution in northern and central Europe. It occurs in dense forest stands, at low elevations in the north, at high elevation in the south (alpine and Carpathian zones).

This is a monoecious, wind-pollinated species. Considering the large area it covers, this is a major species in terms of its ecological and economical importance. Important deterioration due to acid rains has taken place in central Europe, and genetic conservation measures must be taken urgently. Old international provenance experiments exist (established in 1938 and 1968), and results from old progeny tests are also available in different countries, providing interesting data for a scientific basis for plabnning a suitable conservation strategy.

Cork oak *(Quercus suber)*

This is an evergreen hardwood with a mediterranean distribution. The species is economically very important in the areas concerned (Sardinia, Southern Spain and Portugal, North-western Morocco). The species is now subject to complex decline in most of these regions. It is a monoecious wind-pollinated species, with heavy seeds closely dispersed around the parent trees. Although few data are now available about the structure of the genetic diversity of this species, urgent conservation measures must still be taken. Close association with more advanced genetic studies performed on *Quercus ilex* and *Quercus petraea* is recommended.

Noble hardwoods

Several forest species are ordinarily covered by this term. They are all valuable in terms of the quality of the wood, especially for furniture: *Prunus, Sorbus, Malus, Pyrus, Acer, Ulmus, Fraxinus* Most of them are threatened in the light of the general trends in the European sylviculture (endangered wild *Rosaceae*) or disease in the case of *Ulmus (Ceratocystis ulmi*).

But this group is biologically and genetically very heterogeneous,. The most original case study, on wild rosaceous tress concerns the species already selected for the first three gene conservation networks and characterized by their scattered spatial distribution, insect pollination and possible co-evolution with insects, birds and small mammals (because of their fruits).

The conservation programme of the black poplar will be presented in more detail.

A case study on the Black poplar (*Populus nigra)*

Motivation and brief history of the Populus nigra *network*

The choice of *Populus nigra* as one of the pilot networks resulted from the inquiry made under Resolution 2: many countries felt it necessary to share a concerted programme on the conservation of *P. nigra*, but their motivations may vary. We can define three levels of threats to *P. nigra* genetic diversity: anthropogenic alteration of the ecosystem, population extinction, and genetic erosion. Firstly, in all European countries, the native riparian ecosystem is submitted to strong competition with agriculture and forestry (including poplar cultivation), industry, and other human activities. In some cases, the hydraulic engineering needed for the control of flooding may alter the natural disturbance regime, and the ecosystem becomes less favourable for the regeneration of colonising species. These processes affecting the whole ecosystem lead to a progressive destruction of native *P. nigra* populations, or, at least, to the fragmentation of the species distribution. This constitutes the second point: black poplar populations are almost extinct in some marginal parts of the species' area (i.e. Belgium, The Netherlands, UK). In those countries, the species only occurs asisolated individuals and small groups of trees; even, in the UK, the sex ratio among remaining native trees seems highly distorted in favour of males, which might be the result of human selection against cotton producing individuals, and which obviously leads to a reduction of the effective population size. The extinction of such marginal populations could result in the loss of particular adaptations. The third level of threat may be characterised under the general term of genetic erosion and it concerns the whole area of the species. The previously mentioned fragmentation of the species distribution could contribute to that erosion, but the main fear concerns the genetic pollution of native stands by a reduced number of clonal cultivars, either interspecific cultivated hybrids (true introgression from exotic genomes) or *P. nigra* ornamental clones (intraspecific « pollution »): the small number of possible contaminant genotypes and their enormous individual distribution might also reduce the effective population size. The first two levels of threat are clearly evident, even if we often lack a precise evaluation of the process based on repeated inventories, but the real impact of the third threat has not yet been estimated, although the consequences for practical *insitu* conservation are important (are gene resource conservation and poplar cultivation really incompatible? on what scale?).

Another important consideration is the economic interest of the species: the *P. nigra* genome is involved in most of the European poplar cultivars, providing rusticity, good rooting ability, and resistance to bacterial and viral diseases for the interspecific hybrids. In the particular case of Turkey, pure *P. nigra* cultivars represent half of the poplar wood production. The history of *Populus* breeding in Europe goes back to the 18th century, and each poplar breeding institute has its own experience of *P. nigra* germplasm management, generally on an *ex situ* basis (Cagelli & Lefèvre 1997, this volume). The idea of a network for *P. nigra* conservation was a recommendation from the International Poplar Commission (FAO) in 1992. The first official meeting of the EUFORGEN network took place in Turkey in 1994 (Frison & al. 1995); a second meeting took place in Italy in 1995 (Turok & al. 1996), and the most recent was held in Hungary (1996). Twelve countries actively participate in the network (Austria, Belgium, Bulgary, Croatia, France, Germany, Hungary, Italy, The Netherlands, Spain, Turkey, UK), and meeting reports are widely disseminated.

Populus nigra *as a model species for forest gene resource conservation*

Forest tree species are generally recently domesticated, but for black poplar we can define a wild/cultivated complex gene pool analogous to some agricultural crops (Cagelli & Lefèvre 1997, this volume): the cultivated pool is genetically differentiated (interspecific hybrids, which can hybridise with native *P. nigra*), and it is characterised by a very restricted genetic basis (clonal cultivars). *P. nigra* is a pioneer, colonising species of riparian forests. Different kinds of occurrence are observed, from scattered individuals to huge monospecific or mixed stands depending on the local situation. It is a dioecious and wind-pollinated species. Seeds may be transported through wind and water: their viability is particularly short, and strict environmental conditions are required for their germination. The species may also propagate by various asexual means: stem cuttings (broken branches) or root suckers. Gene flow within the gene pool is potentially important due to the biology of the species, but also to the characteristic perturbations of the open ecosystem. Therefore the concept of a population that is perfectly suited to the tree species of the 'climax' forests (*Fagus*, *Abies*), should be replaced, for *Populus nigra*, by the concept of metapopulation. For *in situ* conservation we lack basic information on the species itself. Some interesting results for gene sampling and conservation were obtained from a preliminary study of native black poplar diversity in France (Legionnet 1996): as expected for such a species, the genetic variation was found mainly within stands, but a certain level of differentiation, in particular for non-neutral traits, should not be neglected; the number of vegetative copies of any single genotype seemed to be very limited in adult stands (very little redundancy); and the regeneration sites in a given stand appeared to be genetically structured in space and time.

The riparian ecosystem is also complex, with various components presenting different phases of the sylvigenesis, and its own dynamic where the disturbance regime varies in space and time. Comparatively more work has probably been done on the ecosystem itself: functional typology, the effect of disturbance on regeneration, biogeochemical cycle, etc. In that case, obviously, the whole riparian ecosystem has to be considered for the definition of *in situ* conservation. From these biological considerations the network decided first to set up the basis for an efficient *ex situ* conservation strategy, and then start consisdering an original *in situ* strategy that could be adapted for that species.

Current orientations

Two levels of co-ordination between European activities for *P. nigra* conservation were defined: the short term objective is to co-ordinate the on-going *ex situ* conservation and provide guidelines for those countries willing to start such an activity; the mean term objective is to define a relevant *in situ*, or at least dynamic, strategy of conservation at the European level. The network intends to promote any project for *P. nigra* conservation, either national or concerted international proposals, and, for that purpose, a list of research priorities has been established. In addition, the meeting reports include different documents: annual reports of the national situations, thematic literature reviews, updated bibliography, etc. (Turok & al. 1996).

Based on the experience of both network members and IPGRI, a database for passport data of the European collections is presently under preparation, and will be made available in hard copy and on diskettes. For a standardised characterisation of these collections, a reference collection of 30 *P. nigra* clones from 15 countries has been propagated and will be disseminated to be used as common controls. A list of morphological descriptors for clonal accessions has been adapted to *P. nigra*, on the basis of general UPOV and FAO descriptors for *Populus* taxa. For future collections, a list of stand descriptors will be established. The question of introgression is often addressed when preparing new prospecting or inventories: an illustrated sheet for species identification has been published. The problem here was to give some indications for detecting *in situ* possibly introgressed individuals (that should be checked afterwards) without restricting artificially the variation that may be observed within the 'true' species. A guideline for safe management of clonal *ex situ* conservation, and a literature review on the most recent results for pollen and seed conservation have been published. For the *in situ* strategy, the only real achievements uptonow deal with the conservation of biological reserves that indirectly concern *P. nigra*: a synthesis of the status of the various possible reserves in Europe (objective, ownership, management, control) has been prepared.

Five research needs were given priority, resulting in particular in a research project submitted to the European Union. The actual genetic diversity and its structurate have to be clarified at the European level: this will cover the collection of new germplasm, is necessary for the definition of the European *ex situ* strategy, and is needed to follow-up the evolution of the situation. Another point is that natural regeneration for such a pioneer species involves many factors that should at least be understood for the definition of an efficient *in situ* strategy: from floral biology, migration and gene flow, to regeneration itself and interactions with abiotic factors of the environment. Then, the taxonomy of *P. nigra* needs to be clarified and the real impact of introgression has to be assessed. New technologies for long term conservation should not be neglected for forest trees, especially poplars, that generally support many biotechnologies: this might offer new opportunities for reducing the cost of conservation in space and time. Finally, socio-economical and cultural aspects of *Populus nigra* conservation should be investigated, since the poplar is sometimes 'controversial'.

Perspectives and milestones

For *Populus nigra*, the co-ordination of existing *ex situ* conservation programmes at the European level is well developed. However, this first step remains static in terms of recombination and natural or directed selection. Since *P. nigra* is involved in many poplar breeding programs, coordination of the breeding populations (following a recurrent selection scheme) would be an attempt to establish dynamic *ex situ* conservation: more generally, a dynamic *ex situ* strategy of that kind might be more efficient in terms of creation of diversity, and better controlled, although probably much more expensive, than *in situ* management of a colonising species in a disturbed environment.

Nevertheless, we must propose an *in situ* strategy for *P. nigra* on a large scale, and this cannot be considered without a global approach to the riparian ecosystem; some projects for re-establishment of riverside forests are under study in Belgium, France, Hungary and UK.

Research should provide germplasm managers with a tool for risk evaluation. We need to evaluate the risk of genetic erosion, not only to define a strategy for conservation, and follow it, but also to be able to evaluate the consequences of various human activities on the ecosystem, following the concept of sustainability. Special attention should also be given to the public awareness about needs, objectives and strategies of forest genetic resources conservation.

The European Forest Genetic Resource Programme (EUFORGEN)

According to Resolution 2 of the Strasbourg Conference, IPGRI (International Plant Genetic Resources Institute) and FAO (Forestry Department) jointly proposed a European Forest Genetic Resource Programme, to the second Ministerial Conference on the protection of Forest in Europe, held in Helsinki on June 1993.

This project was officially accepted and its funding process was begun. The technical content goes further than the four pilot forest gene conservation networks initiated by the Follow Up Committee of Resolution 2 of the Strasbourg Conference, previously described. This programme will insure a permanent follow up of the conservation of the main European forest gene resources, and a continuous stimulus for more basic research into conservation strategies, as well as the development of cooperative forest germplasm and data banks.

The coordinating structure will be composed of an Executive Committee involving IPGRI and FAO, and a Steering Committee formed of the national representatives.

Each State adheres to the programme on a voluntary basis. The financial participation depends upon economic importance of the State, the number of species of concern and later on the level of involvement of the national Institutions.

Conclusions

Four pilot gene conservation networks are now under way concerning Norway spruce, cork oak, black poplar and noble hardwoods. These gene conservation projects will be developped through an evolvinge EUropean FORest GENetic ressource programme (EUFORGEN, Turok 1995). Greater efficiency in these gene conservation projects requires more research into dealing with: analysis of the structureof the genetic variability of these species, the study of the mechanisms involved in the maintenance of a high level of genetic diversity in the natural forests, the breeding systems and effective population sizes. The effects of sylvicultural and forest management practices on the long term evolution of the genetic diversity of the forest tree populations also needs to be considered. Similarly, the application to forestry of methods already used with cultivated plants, such as 'core collections' (Brown 1989), and dynamic management of *ex situ* conserved genetic resources, would be highly desirable.

We have also to integrate further the effects of expected global climatic changes into our conservation strategies, and extend them to minor and rare forest tree species. More efforts on data analysis of the oldest multi-site provenance trials will help us gain a better understanding of what happens genetically with long distance seed transport. The setting up of conservation networks dealing with some representative samples of forest ecosystems will probably also help us with the genetic conservation of non economic forest species.

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