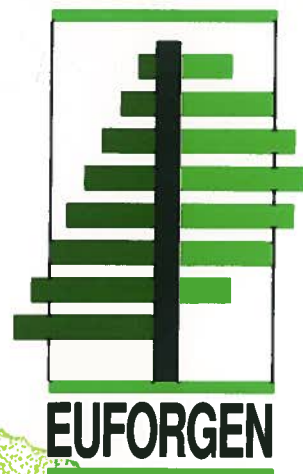


EUROPEAN FOREST GENETIC RESOURCES PROGRAMME (EUFORGEN)

# *Populus nigra* Network

Report of the third meeting  
5-7 October 1996  
Sárovár, Hungary

**J. Turok, F. Lefèvre, S. de Vries** and **B. Tóth**, compilers



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The International Plant Genetic Resources Institute (IPGRI) is an autonomous international scientific organization operating under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The international status of IPGRI is conferred under an Establishment Agreement which, by January 1997, had been signed by the Governments of 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, Slovak Republic, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine. IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI works in partnership with other organizations, undertaking research, training and the provision of scientific and technical advice and information, and has a particularly strong programme link with the Food and Agriculture Organization of the United Nations. Financial support for the research agenda of IPGRI is provided by the Governments of Australia, Austria, Belgium, Canada, China, Denmark, Finland, France, Germany, India, Italy, Japan, the Republic of Korea, Luxembourg, Mexico, the Netherlands, Norway, the Philippines, Spain, Sweden, Switzerland, the UK and the USA, and by the Asian Development Bank, CTA, European Union, IDRC, IFAD, Interamerican Development Bank, UNDP and the World Bank.

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.

The geographical designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of IPGRI or the CGIAR concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. Similarly, the views expressed are those of the authors and do not necessarily reflect the views of these participating organizations.

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## Third meeting of the EUFORGEN *Populus nigra* Network, a pioneer network for a pioneer tree species: growing up

*François Lefèvre, Chair and Sven M.G. de Vries, Vice-Chair of the Network*

Prior to the establishment of the EUFORGEN Network, the advisory group of experts on *Populus nigra* genetic resources stated in February 1993:

"Following the recommendations from the 19th Session of the International Poplar Commission (held in Spain, 1992), and in the framework of Strasbourg Ministerial Resolution S2, several European countries will collaborate in a conservation network on *Populus nigra*. An *in situ* conservation strategy will probably not be easy to define for this species, because riparian sites are much disturbed by human activity; interspecific hybrids are cultivated in the ecological zone of the natural stands; and this ecological zone is largely open to geneflow through seeds and pollen. In the urgency, the already existing *ex situ* conservation programmes will be coordinated. Meanwhile, the participants will initiate an *in situ* conservation network based on the present knowledge."

Following two previous meetings in Izmit, Turkey and Casale Monferrato, Italy, the third meeting of the Network was held 5-7 October 1996 in Sárvár, Hungary. The meeting was attended by participants from 12 countries (see List of Participants). It was organized by the Hungarian national programme, in conjunction with the 20th Session of the International Poplar Commission of FAO, which was held in Budapest from 1 to 4 October 1996. The Network meeting was opened by Dr G. Holdampf of the Ministry of Agriculture from Budapest and Prof. E. Führer, Director General of the Forest Research Institute. They welcomed the participants and wished them a successful meeting. Prof. C. Mátyás, the national coordinator of forest genetic resources activities, stressed the role of efficient international collaboration for implementing gene conservation strategies in each country. Several representatives of the forest service, research institutions and nature-protected areas in Hungary attended the meeting as observers.

The discussions during the meeting were divided into two major parts: updating and further planning of common tasks of the workplan as well as discussing the development of gene conservation strategies for the species (see Programme).

After the meeting in Sárvár, the following observations were made: (1) a fair level of coordination in the Network has been achieved whenever the scientific basis for an optimal conservation strategy was available, in particular for *ex situ* conservation; (2) collaborative work is emerging in a number of areas and the participants from newly attending countries join the workplan tasks developed so far by the Network; (3) a constructive discussion is in progress for issues which still need research work, in particular the practical management of *in situ* conservation.

The practical achievements of the Network are listed below (see Workplan):

- Sixteen countries have actively participated in the Network so far, and national reports were published. A first synthesis has been made concerning the context for *in situ* conservation (see this volume). Further general synthesis is planned for updated reports;

- For inventories, our identification sheet seems to be very *pop(u)lar*, and is practically used to detect possible introgressive forms. A standardized description of native poplar stands is being prepared;
- For *ex situ* collections, practical guidelines for safe conservation in the field or as seed banks were produced;
- A database of the European collections is established, including passport data, and will be annually updated. Before the meeting, 1651 clones from 5 countries had been stored in the database, and it was found efficient for detecting the duplications among collections, or identifying accessions from a country;
- For the characterization of the national collections, a standardized list of descriptors (see this volume) was produced and a set of 15 reference clones from unique stool-beds disseminated among the participants;
- A EUFORGEN core collection of native *P. nigra* has been established and 15 countries provided clones; others are expected from the whole distribution range. This collection will be distributed among the participants later, as a common set of clones to which the evaluation of national collections could refer. Preliminary observations reveal high levels of morphological variation among the clones;
- A list of references related to *P. nigra* germplasm conservation is being annually updated (see this volume);
- For public awareness, which is considered as an important objective, audio-visual aids are prepared using slides and videos from the Network participants;
- Research priorities are defined, and a joint proposal has been submitted for funding to the EU;
- The most recent scientific results are turned into practical conclusions, e.g. guidelines for seed storage (see this volume).

According to the national reports (see this volume and previous Reports of the Network meetings), all these outputs provided by the EUFORGEN Network seem to be an effective contribution to supporting research and implementing applied conservation at both the national and the European levels.

An efficient strategy for gene resource conservation of black poplar needs to integrate several approaches: *ex situ*, *in situ* and dynamic population management. For the coming years, our efforts should focus on developing the methodology for *in situ* conservation. The focus will shift from the concept of 'natural monument' to the management of protected areas, or even the re-establishment of riparian forest. This is not an easy task, owing to the biology of the species and the characteristics of the riparian ecosystems, and also to the 'administrative diversity' of institutions responsible for *in situ* management. One of the continuous tasks of the Network is to identify the most pressing research needs.

During the third meeting of the Network, the population genetic viewpoint of *P. nigra* gene conservation was given particular emphasis (see Legionnet, this volume). As expected for a dioecious forest tree species, a fair level of genetic diversity is found with isozyme markers, mainly at the within-stand level; but some results suggest that geneflow among stands might be restricted, allowing for a certain level of differentiation for adaptive traits. The question of geneflow should be further investigated in order to optimize the sampling strategies within and between stands. In the same area, for example, the increase of diversity in older age

classes should also be taken into account when collecting material. Beyond the immediate question of sampling the diversity, given its present structure and past history, we need to predict its evolution in order, at least, to be able to assess the risks caused by anthropic factors. The metapopulation model should be taken as a useful reference for investigating these processes. Preliminary results of research were presented and discussed by the participants, but further investigations are needed in the fields of demography and population dynamics (migration, extinction, etc.). These research areas concern both geneticists, at the species level, and ecologists for the ecosystem. The evolution of genetic diversity is also determined by the genetic load and inbreeding depression. Their importance should be evaluated for *P. nigra* since they influence the susceptibility of the species to the fragmentation of populations which has steadily increased because of human activities.

Another question debated was introgression, which is not "natural" for *P. nigra* but is due to the cultivation of exotic hybrid poplars. One can consider that the species needs to be conserved as an entity belonging to the overall biological diversity, and therefore attention should be paid to the risk of artificial modification of the natural genepool. In this case, it is a great responsibility to deal with the genetic implications of human activity (poplar cultivation). This is an ethical point of view. From another point of view, one can consider that introgressive types may be badly adapted owing to the breakdown of co-adapted gene complexes, in which case they do not represent a real threat for the species as a whole unless most of the natural regeneration occurs after genetic pollution. Introgression might even be considered to represent a chance for the species to increase its genetic diversity. This seems to be a more pragmatic point of view. It also sounds like a more fundamental debate between the biodiversity conservation approach based on taxonomic units, and the genetic approach which rather considers the complex genepool and its evolution. Beyond our discussions, the level to which we care about introgression in the *in situ* management may have important economic consequences: should we regulate the cultivation of hybrid poplars somehow? One conclusion was that we need more objective information about the effective amount of introgression and its possible effects in the long term.

The issue of broadening the scope of the Network was also addressed during the meeting. The decision, however, was postponed to the next meeting, since much work is currently underway with *P. nigra* and efficiency is required. Participants agreed that broadening could eventually concern *P. alba*, one of the species also identified during the follow-up process to Resolution S2.

The participants expressed their gratitude to Hungarian colleagues, particularly to Prof. B. Tóth, Honorary Chairman of the Hungarian Poplar Commission, for kindly offering and providing local organization of this meeting.

François Lefèvre was unanimously re-elected Chair and Sven de Vries Vice-Chair of the Network for the next period of two years. It was agreed that a two-year period can be prolonged only once. Jos Van Slycken offered to host the next Network meeting in Geraardsbergen, Belgium, in October 1997. His offer was appreciated by all participants.



## Workplan update<sup>1</sup>

### Exchange of reference clones

**Coordinator:** J. Van Slycken

**Objective:** to develop a list of well-known clones and circulate material to all interested countries for regeneration in their stool-beds and for comparison with the respective national collections

**Status:** material sent to 10 countries in 1995

**Action and deadlines:** Interested participants from newly attending countries will request the reference clones from J. Van Slycken by the end of 1996. He will distribute the material in February 1997.

### EUFORGEN core collection of clones

**Coordinator:** L. Cagelli (S. Bisoffi)

**Objective:** to set up a common basis for the characterization and evaluation of national clone collections

**Status:** 15 countries sent cuttings in 1995 but the rate of success was very low and information in several cases incomplete

**Action and deadlines:** Detailed requirements and conditions for the shipment of cuttings (at least 25 cuttings from each of the two representative clones per country), as agreed at the previous Network meeting, will be prepared by L. Cagelli and distributed to the concerned participants by J. Turok before 1 November 1996. J. Turok will contact potential donors (and recipients) of the collection from all other countries in the distribution area by 15 November 1996. Participants of the meeting will send the contact addresses of colleagues working with *P. nigra* they have in any of these countries to J. Turok before 1 November 1996. A reminding letter will be sent to all contributors before 15 January 1997. The material will ultimately be sent to each interested country on request.

### Passport data for the database

**Coordinator:** L. Cagelli

**Objective:** to create a tool for common activities including database, inventories and exchange of material

**Status:** published in 1. Report and updated later

**Action and deadlines:** The updated version will be sent to each participant from the newly attending countries along with the draft report of the meeting before 15 November 1996 and will then be published in the final Report. J. Turok will receive a German translation from B. Heinze and will ensure the translation into French and Russian before 1 November 1996. The translated versions will be sent to the relevant countries.

### European database

**Coordinator:** L. Cagelli (S. Bisoffi)

**Objective:** to have an efficient tool for information management, exchange of clones, detection of duplications among national collections and for better identification of accessions

**Status:** files received so far from five countries

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<sup>1</sup> This Workplan update results from, and was distributed at, the meeting.

**Action and deadlines:** S. Bisoffi will provide the existing database to J. Turok who will ensure its inclusion on the Internet before the end of the year 1996. Each interested participant will send a file according to the agreed passport data by 1 February 1997. The minimum information is the clone name/number. Also foreign clones should be mentioned, as long as the original clone name/number is available. The inclusion of data, however, is restricted by the availability of existing accessions (cuttings, pollen, or seeds) except for the parents of available accessions which should be included (if maintained or not). For updating, a complete country file should be sent to replace completely the original one.

#### **Descriptor list for *P. nigra* clones**

**Coordinator:** J. Van Slycken

**Objective:** to produce common minimum standards for characterizing clone collections in each country

**Status:** published in 2. Report

**Action and deadlines:** none

#### **Standardized minimum list of descriptors for inventories of *P. nigra* stands**

**Coordinators:** M. Hofmann and N. Alba

**Objective:** to produce common minimum standards for characterizing and monitoring *in situ* genetic resources

**Status:** new task

**Action and deadlines:** A preliminary list was presented, briefly discussed and distributed at the meeting. Participants will send their comments and suggestions to N. Alba before 1 February 1997. N. Alba and M. Hofmann will develop an advanced version for discussion and approval at the next meeting.

#### **Identification Sheet**

**Coordinator:** J. Turok

**Objective:** to facilitate the simplest possible identification of *P. nigra* from cultivated hybrids and possible introgressive forms in the field

**Status:** published along with 2. Report

**Action and deadlines:** Possibilities to produce the Identification Sheet in different European languages will be investigated by J. Turok. He will inform all participants about the production schedule and will ask for the translated versions by 1 February 1997.

#### **Synthesis of *in situ* gene conservation measures and activities**

**Coordinator:** S. de Vries

**Objective:** to assess the current status of measures used and activities undertaken for *in situ* conservation of genetic resources of *P. nigra*

**Status:** to be published in 3. Report and updated afterwards

**Action and deadlines:** A table was presented and distributed during the meeting. All participants were asked to provide an input on their national activities and send the table back to S. de Vries by 1 November 1996. S. de Vries will compile the table for publication in the Report of the meeting (to be sent to J. Turok by 10 December 1996).

**Guidelines for *ex situ* field collections**

**Coordinator:** S. de Vries

**Objective:** to provide a practical guide for the maintenance of *ex situ* field collections

**Status:** published in 2. Report

**Action and deadlines:** none

**Guidelines for *ex situ* gene conservation methods**

**Coordinator:** L. Cagelli (S. Bisoffi)

**Objective:** to provide a practical guide for *ex situ* conservation of seeds and pollen, based on experimental data

**Status:** to be published in 3. Report

**Action and deadlines:** S. Bisoffi will send the document to J. Turok for publication in 3. Report before 10 December 1996. J. Turok will ensure the inclusion of final Guidelines on the Internet before the end of the year 1996.

**Review of literature**

**Coordinator:** F. Lefèvre

**Objective:** to provide regularly updated literature reviews for use by the Network

**Status:** published in 1. and prepared for publication in 3. Report

**Action and deadlines:** F. Lefèvre will send the file to J. Turok by 10 December 1996, after having received corrections and additional references from the participants (before 1 November 1996). J. Turok will ensure input from the national focal point in the Russian Federation. This item output will also be available through the Internet.

**Public awareness**

**Coordinator:** S. de Vries

**Objective:** to produce a universal collection for use by the Network members

*Collection of slides:* More than 100 slides have been collected so far from four countries. Missing topics were identified and participants were requested to send their slides with captions to S. de Vries before 1 February 1997. J. Turok will find out the possibilities for the production of public awareness media in IPGRI. Among the missing topics were mentioned:

- riparian ecological succession
- wood patterns due to burls
- buttresses
- natural monuments/individually protected trees
- re-establishment of the riparian ecosystem
- fodder for animals
- particularly interesting landscapes (Hungary, North Africa)
- biotechnologies
- pollen
- paintings, art
- Identification Sheet.

*Leaflet production:* After the slide collection has been completed, a leaflet will be produced to inform about its use and possible presentation modes.

**Country reports and updating of information**

**Coordinator:** F. Lefèvre

**Objective:** to ensure regular exchange of information among the countries and to monitor the impact of international activities at the national level

**Status:** regular task

**Action and deadlines:** Each participant will send either an update or introductory report on the national activities by 1 November 1996 to J. Turok for inclusion in the Report of the meeting. A review article has been planned for a later stage.

**Molecular methods available for the characterization of *P. nigra***

**Coordinator:** B. Heinze

**Objective:** to review and develop modern methods for characterization of the existing collections

**Status:** new task

**Action and deadlines:** As a first step, an overview of the existing methods will be prepared for the next Network meeting. It is intended that molecular markers be used for genetic characterization of the EUFORGEN clone collection, and to test their performance on pedigrees from controlled crossings.

**Submission of joint research project proposals**

**Coordinator:** S. de Vries

**Objective:** to launch a collaborative research framework on genetic diversity of the species

**Status:** regular task

**Action and deadlines:** The new proposal will be prepared for submission to the next call (deadline in March 1997).

It was also agreed that each country indicates the mailing addresses and numbers of requested copies for the Network publications to be sent by IPGRI.

## Passport data for *Populus nigra*

**Luisa Cagelli**

Istituto di Sperimentazione per la Pioppicoltura, 15033 Casale Monferrato, Italy

### 1. Clone name/number

Name or number assigned when a clone is entered into the collection. It serves as a unique identifier and is assigned whenever a clone is entered into the collection. Letters should be used before the name/number to identify genebank or national system:

ISP\_N001            for the material maintained at ISP (Casale Monferrato, Italy)  
 ISP\_Brisighella    for the material maintained at ISP (Casale Monferrato, Italy)  
 INRA\_71002        for the material maintained at INRA (Orléans, France).

In this way codes used by the different institutes involved in *P. nigra* genetic conservation (N001 for ISP Casale Monferrato, 71002 for INRA Orléans, etc.) can be preserved with letters before the clone name/number to identify institutes that maintain a clone.

It is important to use letters that identify the institute and not just the country, because in some countries there might be more than one institute involved in genetic conservation.

### 2. Country where maintained

Country in which clone is maintained. International three-letter standard abbreviations (codes) should be used, according to ISO 1988, N. 3166.

### 3. Institution where maintained

Name of institution in which clone is maintained. Abbreviations adopted to identify institutions under "Clone name/number" should be used.

### 4. Original clone name/number

Codes assigned from the institution of origin.

This is very important when the clone was obtained from another institution. It could be useful to identify duplicates held in different collections and should always accompany samples wherever they are sent.

### 5. Collecting number

Original number assigned by collector of the sample.

This item could be useful to identify duplicates held in different collections and should always accompany samples wherever they are sent (this number may be different from the "Original clone name/number").

**6. Other name/number associated with the clone**

Any other identification numbers known for this clone.

- 6.1 Other name/number 1
- 6.2 Other name/number 2
- 6.3 Other name/number 3
- 6.4 Other name/number 4
- 6.5 Other name/number 5

**7. Type of maintenance**

- 0. None
- 1. Nursery stool-bed
- 2. Adult plantation
- 3. Pollen
- 4. Nursery stool-bed and adult plantation
- 5. Nursery stool-bed and pollen
- 6. Adult plantation and pollen
- 7. Nursery stool-bed, adult plantation and pollen

**8. Notes**

Any additional information should be specified here.

**9. Collecting institution**

Institution which originally collected the sample.

**10. Country of origin**

Country in which the sample was collected. International three-letter standard abbreviations (codes) should be used, according to ISO 1988, N. 3166.

**11. Province/State**

Name of the primary administrative subdivision of the country in which the sample was collected.

**12. Department/County**

Name of the secondary administrative subdivision (within a Province/State) of the country in which the sample was collected.

**13. Location of collecting site**

Name of the nearest town or village where the sample was originally collected.

**14. Collecting date of original sample**

Year in which the sample was originally collected (YYYY).

**15. Latitude of collecting site**

Degrees and minutes followed by N (North)  
(e.g. Casale Monferrato 45°07' North: 04507N)

**16. Longitude of collecting site**

Degrees and minutes followed by E (East) or W (West)  
(e.g. Casale Monferrato 8°30' East: 00830E)

**17. Elevation of collecting site**

Altitude above sea level (m).

**18. Sex**

1. Female
2. Male

**19. Female parent**

When the female parent is known (clone obtained from artificial crossing or from seed collected from known genotypes), the "Original clone name/number" of the female parent should be specified. Include the parents in the database even if they are not currently present in stool-beds (the "Type of maintenance" should then be "none").

**20. Male parent**

When the male parent is known (clone obtained from artificial crossing), the "Original clone name/number" of the male parent should be specified. Include the parents in the database even if they are not currently present in stool-beds (the "Type of maintenance" should then be "none").

### General rules for compilation

**Material to be included in the database**

As poplar is propagated by means of cuttings it was decided to include in the database only the clones that are propagated in national collections. Three main types of clones should be included:

- clones collected in the national territory
- clones used as parents in controlled crosses: it is important to include the parents of the clones included in the database even if the parents are not currently present in stool-beds (in this case specify "none" in "Type of maintenance"), so as to have some information about the origin of the clones obtained by open-pollination or from artificial crossing
- clones received from other countries if the original name is known.

**Type of sample originally collected**

Although, as already specified, only clones must be included in the database, two different types of samples might have been originally collected: cuttings, if the clone derives from vegetative propagation of a spontaneous tree, or seed if the clone derives from seed collected from a spontaneous tree or from artificial crosses. It is important to make this clear because a lot of the information to be included in the passport database is related to the original sample (collecting number, collecting institution, country of origin, province/state, department/county, location of collecting site, collecting date of original sample, latitude, longitude and elevation of collecting site).

**Exchange of data**

It is important to follow some specific rules so that the information from all the EUFORGEN members will be uniform.

**Characters**

Only the 26 CAPITAL LETTERS of the English alphabet should be used. This is very important because some particular accented letters existing in some alphabets are not available in computers.

### File format

The information to be included in the database must be forwarded in a file in ASCII format. The size of the fields is indicated in Table 1. If more characters are necessary for some particular fields (e.g. the field "Notes" reserved for additional information), they can be enlarged but with specification in the note. If there is more than one word in one field it is necessary to use an underscore (\_) between the words (e.g. CASALE\_MONFERRATO).

Alternatively, the information to be included in the database can be forwarded in a file in EXCEL format.

### Missing data

Dash (-) should be inserted for every missing data: in some cases it will probably be possible to complete the data later (sex, etc.).

### Empty fields

Dash (-) should be inserted also in those fields which are completely empty (such as, probably in many cases "Other name associated to the clone") so as to have the same file structure from every partner.

### Geographic coordinates

The programme Arcinfo for the conversion of geographic coordinates used by different countries can be used. Every participant shall introduce latitude and longitude in the database according to the projection system adopted in the respective country (specifying in a note which system was adopted, e.g. in Italy the system used is UTM). It will be possible to convert the coordinates later if necessary.

**Table 1. Size of fields (maximum number of characters for each field).**

No.	Field name	Max. number of characters
1	Clone name/number	20
2	Country where maintained	3
3	Institution where maintained	15
4	Original clone name/number	20
5	Collecting number	20
6	Other name/number associated with the clone (1)	20
	Other name/number associated with the clone (2)	20
	Other name/number associated with the clone (3)	20
	Other name/number associated with the clone (4)	20
	Other name/number associated with the clone (5)	20
7	Type of maintenance	2
8	Notes	100
9	Collecting institution	20
10	Country of origin	3
11	Province/state	20
12	Department/county	25
13	Location of collecting site	25
14	Collecting date of original sample	4
15	Latitude of collecting site	6
16	Longitude of collecting site	6
17	Elevation of collecting site	4
18	Sex	2
19	Female parent	20
20	Male parent	20



## Guidelines for seed and pollen storage

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Pollen and seed storage is considered an alternative method for the *ex situ* conservation of genetic resources. The establishment of collections of pollen and seed represents in fact the most economical and easiest way to keep a very large genetic diversity. However, for pollen and seed collections to be used efficiently it is important to define the best conditions for their long-term storage.

### Seed

Poplar seeds are very small (1000 seeds weight about 1 g) and seed germination decreases rapidly in 3-4 weeks.

Many factors affect the variability of germination over time: the time of collecting, the period between collecting and the beginning of storage, the moisture content of seed and the temperature of storage.

First of all it is better to collect seed as near as possible to its natural ripening period, when the fruits begin to open; if prematurely collected, the seed will not ripen and viability will be poor.

The period between collecting and the beginning of storage must be as short as possible: the fruits have to be placed on a thin layer at room temperature and the seed must be extracted and separated from the cotton (using sieves and an air stream) within 1 week of collecting.

Dehydration is a very important factor for storage: the seed can be stored successfully for some years at low temperatures if the moisture content is reduced to 6-8%. Sometimes black poplar seed reaches this moisture content naturally without any treatment. Otherwise the moisture content can be reduced in the oven at about 35°C for a short time (about 10-30 minutes depending on the initial moisture content).

As for storage temperature, good results have been obtained at -18 to -40°C. As no differences of germination rate have been observed in this range, a temperature of -18°C, which can be achieved in a normal home freezer, can be suggested for long-term preservation of viability.

### Pollen

As with seed, pollen viability is affected by many factors: the time and method of collecting, the period between collecting and the beginning of storage, the moisture content of pollen and the temperature of storage.

However, whereas seed viability can be easily tested with reliable germination tests, with pollen things are not so easy. In fact, although viability can be evaluated with different methods, there is not enough information regarding the relationship between viability and seed-setting ability.

Although male floral branches can be collected over all the winter period, generally a large amount of pollen is obtained from branches collected as near as possible to natural ripening and put in water pots in the greenhouse (about +20°C; 70% relative humidity).

Pollen can be collected in two different ways: directly when falling from the anthers or from completely developed catkins collected before the anthers open and put on a sieve for 24 hours (at about +25°C; 40% relative humidity).

As with seed, dehydration seems to be a very important factor for pollen storage: the pollen moisture content should be reduced to about 10%.

Also as with seed, low temperatures (-18 to -40°C) are essential for long-term storage.

Further studies are necessary to define the optimum moisture content and the best storage temperature. However, in many cases pollen stored at -40°C for up to 4-5 years was used successfully for crossings; the seed obtained generally showed good germination capacity.

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## An overview of *in situ* gene conservation measures

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One of the methods for preserving genetic diversity in the long term is dynamic conservation of genes *in situ*. With *Populus nigra* as a pioneer tree species of European riparian forest, an overview of the currently applied measures is presented taking into account the different *in situ* gene conservation management systems in different countries.

Information was gathered from the participants of 13 Network countries with regard to the measures, methods and regulations for *in situ* conservation. Most data were extracted from country reports presented during the three Network meetings. Since some contributions are more detailed or differently focused than others, the overview has a preliminary and in some parts probably balanced character. A table was compiled before the third Network meeting, discussed and improved during that meeting. A blank field means no information is available.

All countries participating in the Network make use of *in situ* conservation in some way. *Populus nigra* still occurs either as scattered individual trees (Belgium and UK) or forms either pure stands of *P. nigra* or is admixed with other species (see Table). Only Spain and France have pure *P. nigra* stands together with individual *P. nigra* trees as well as *P. nigra* in mixed stands.

Re-introduction of *P. nigra* on the banks of rivers is taking place or is planned to take place in several countries (Austria, Belgium, Croatia, Czech Republic, Hungary, the Netherlands, Slovakia and the UK). Larger or smaller areas along the rivers are bought or dedicated for this purpose by either private nature conservancy organizations or by governmental institutions (see Table).

Some countries use natural ways to re-introduce *P. nigra* in such areas (the Netherlands, UK, etc.) while other countries such as Croatia influence this process by seed-harvesting techniques. In both cases *ex situ* collections serve as the basic material for these re-introductions. All participating countries maintain *ex situ* collections, varying from 100 (UK) to 300 (France) accessions. A selection of both male clones and female clones is used for this purpose.

Protection of areas is often based on specific regional regulations. Often there is a general law in which the different kinds of protected areas are described (natural parks, natural reserves, natural monuments or other areas of particular interest). Formalities for their designation, managing board, the instruments for management and the administrative sanctions are indicated.

Most countries have a Forestry Act under which there will be a variety of possibilities to prevent cutting *P. nigra* either as individual trees or as part of plantations through their value, e.g. for the landscape. Only Spain and the UK have specific laws for the protection of *P. nigra* on a species level; other countries are able to protect *P. nigra* either as part of a specific type of forest ecosystem (Austria, Belgium, Czech Republic, Hungary, the Netherlands and Spain) for which Belgium also includes management rules. Most countries only have laws for the protection of certain areas or a specific location (see Table) for which France, Italy, Hungary and the Netherlands also indicate management rules. Austria has put *P. nigra* on the red list in some federal states. This list serves as a basis for legal protection.

Status March 1997

	FR	IT	BE	CR	DE	HU	NL	ES	UK	TR	SK	CZ	AT
<b>Specific legislation for the protection of different aspects and at different levels:</b>													
(a) <i>P. nigra</i> as species	N	N	N	N	N	N	N	N	Y	N		N	N
(b) forest communities	N	N	Y*	N		Y	Y*	Y	N	N		Y	Y
(c) areas/habitats (* = with management role)	Y*	Y*	Y*	Y*	Y	Y*	Y*	Y	N	Y		Y	Y*
(d) conservation practice excludes production due to the registration	Y		N				Y		Y			Y	Y
(e) implemented at the regional level by government (g) or by private owners (p)	g,p	g	g,p		g,p	g	g,p	g	g			g	g,p
(f) protection of individual trees (i) or trees in mixed stands (m) or trees in pure stands (p)	i,m,p	i,m	i	m	i,m	i,m	i,m	i,m,p	i		i,m	i,m	i,m
<b>Re-introduction carried out (or planned - p)</b>	N		p	p		p	Y	N	p		Y	Y	Y
<b>Economic importance of <i>P. nigra</i></b>	N		N	Y		Y	N	N		Y	Y	Y	N
<b>Regulations on planting poplar hybrids in vicinity</b>	N		N			N	N	N			N	N	N
<b>Occurrence of natural regeneration</b>	Y		N			Y	Y	Y			N	N	
<b>Inventories carried out at a national (n) or regional (r) level, or are planned (p)</b>	p	r	n			n,r	n,r	p			n	n	
<b>Availability of maps for the <i>in situ</i> conservation areas</b>	Y	Y	Y			Y	Y	Y			Y	Y	

Y = yes, available; N = no, not available.

FR=France; IT=Italy; BE=Belgium;

CR=Croatia; DE=Germany; HU=Hungary;

NL=Netherlands; ES=Spain; UK=United

Kingdom; TR=Turkey; SK=Slovak

Republic; CZ=Czech Republic; AT=Austria.

In the UK there are no specific measures to safeguard *P. nigra* in an explicit way. Trees generally are protected under the Forestry Act while local authorities can place "Tree Preservation Orders" on trees to prevent their destruction, or to ensure that they are replaced.

No specific laws exist, for instance, in the Netherlands and Italy to regulate the type of material to be (or not to be!) planted in the vicinity of protected areas. Relevant in this situation is the use of hybrid poplars and the use of *P. nigra* var. *italica*, a well-known ornamental male clone.

For the establishment of plantations for the purpose of *in situ* conservation, the objective of conservation excluding commercial production has to be officially recognized in order to be able to grow unregistered material in nurseries. This is regulated in Austria, Czech Republic, the Netherlands and the UK.

In Spain, a specific programme for the genetic conservation of *P. nigra* (and *P. alba*) was recently approved by the Ministry of Agriculture. Similar specific action is not known for any of the other participating countries.

As for the related research activities, all participating countries make efforts towards inventories of *P. nigra* genetic resources at a national level (Belgium, Czech Republic, Slovakia) or regional (Italy) or both (Hungary, the Netherlands). France and Spain are currently planning the inventories. Maps of distribution are available for many areas in these countries. Plant material can thus be reproduced accordingly if required. Some countries indicated having used the descriptor lists developed by the Network for their inventories.

In many countries, biochemical studies also take place in order to find out more about the extent to which *P. nigra* is still available as a pure species and the extent of introgression.

Natural regeneration occurs in France, Hungary, Spain and the Netherlands. Presumably in many more countries too, but more detailed data would be needed for evaluating this development. Belgium, Czech Republic and Slovakia indicated having problems with natural regeneration of *P. nigra* recently.

## Reports on the progress of activities in countries

### Hungary

#### 1. Overview of the progress made in the national gene conservation programme

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After the last meeting of the *P. nigra* Network (held in Casale Monferrato, Italy, in September 1995), the following steps were undertaken in Hungary:

- search of pure *P. nigra* occurrences
- survey of forest subcompartments holding *P. nigra* occurrences and registered in the national inventory of forests
- investigation of species identity and purity
- development of a gene collection
- improvement of the registration and documentation process.

The species description standards developed and agreed by the *P. nigra* Network were used. Furthermore, the registration list compiled for the meeting in Casale Monferrato as a recommendation was found very useful.

Within the activity sphere of Sárvár Research Station (northwestern part of the country), the following activities have been carried out lately:

- Designation of 52 *P. nigra* mean stems in populations preserved under *in situ* conditions. 9 plus trees were taken for *ex situ* conservation.
- Establishment of a new plantation for *ex situ* gene conservation purposes, with the use of rooted plants raised of 33 *P. nigra* clones at 3 localities.
- Contribution to the development of the Network's core collection of clones. After having sent 2 clones to the European collection in Casale Monferrato in 1996, the Sárvár collection is ready to receive clones by the spring of 1997.
- Resistance testing of leaf-attacking pests implemented by the Forest Research Institute.

Furthermore, 18 *P. nigra* individuals were designated for the *in situ* gene preservation in the eastern part of the country and 5 plus trees for *ex situ* conservation.

The introduction of the Network's registration system and particularly the common set of passport data for *Populus nigra* (elaborated by L. Cagelli) started. There is only one difficulty, namely that some data required are missing. The collection of these data is a task ahead of us.

The National Institute for Agricultural Quality Testing (OMMI) in Budapest developed a registration system for the computerized registration of plus trees of forest tree species and clone collections in 1996. The registration data include: owner, taxonomy, morphological characteristics, site description, dendrometric data, genetic evaluation and other features. There are currently 98 *P. nigra* plus trees and 63 clones (preserved in stool-beds) included in that system.

A DNA laboratory has been installed in the National Institute for Agricultural Quality Testing and the first important research task will focus on the genetic

polymorphisms of oaks. The equipment should be suitable for similar examination of the black poplar plus trees and clone collections. This type of investigation will, it is hoped, become part of our *P. nigra* Network research efforts (including RAPD, RFLP and other DNA marker examinations). It would be desirable for foreign institutes with experience in this field to exchange their methodologies with OMMI. In this case the new laboratory would be able to make examinations for the neighbouring countries too.

The Hungarian Parliament enacted a new Nature Protection Law 1996 (see below). This law does not take *P. nigra* under protection as a species, but creates the possibility to protect plant communities with important components of *P. nigra*.

The Hungarian national programme on *P. nigra* gene conservation will continue in the implementation of the aforementioned tasks (such as the inventories/search of pure *P. nigra* occurrences, examination of identity and purity, further development of *ex situ* and *in situ* gene conservation methods, registration and documentation systems, further development of the molecular investigations and, very importantly preservation of *P. nigra* occurrences by practical protection and conservation measures.

A fundamental difficulty in the fulfilment of these tasks is the lack of sufficient financial means. As the country has many financial problems, it is a serious concern that the promising work will slow down or even temporarily stop. In order to avoid such unfavourable development, international support would be necessary (e.g. PHARE projects, EU programmes and other possible sources).

## 2. Conditions of *in situ* conservation of *Populus nigra* in Hungary on the basis of the new Nature Protection Law

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Various complex social problems have arisen in Hungary recently due to political and economical changes. The whole legal system had to be revised by the Parliament in order to modify the economic structure of the country. Three new laws of great importance – the Forestry, Wild Management and Nature Protection Laws – were passed by the Hungarian Parliament in 1996. The new Nature Protection Law states the conditions necessary to promote the *in situ* conservation of endangered species and ecosystems. This brief review interprets the most important and interesting points of this new Law.

Paragraphs of general validity in connection with *in situ* conservation

Based on the fundamental principles of the Law, natural resources in protected areas and sites (biotopes) can only be utilized to such an extent and in such a manner that their survival is not endangered. The Hungarian State cooperates with other countries and international organizations to protect the natural environment. In the absence of international contracts and conventions, the State will respect the basic principles of nature protection (§5 of the Nature Protection Law). When utilizing the natural environment and natural resources, all natural conditions (water, soil, etc.) should be protected and preserved as far as possible (§6). The Ministry of Environment Protection and Regional Development is charged with the

compilation of a general National Nature Protection Programme (NNPP) laying down the general criteria for nature protection. The NNPP must include a strategy for conservation activities (§53). On the basis of the Law, all types of agricultural management conducted in a natural or a nature-friendly manner will be supported and sponsored by the Ministry, as will the reconstruction and (re)forming of autochthonous biotopes (§71).

#### *In situ* conservation in non-protected areas

The Law declares that wild biological organisms and associations of such (which are not used for farming purposes) should be protected together with their sites (biotopes). Black poplar (*Populus nigra*) is not a protected species in Hungary, so it will only be protected when its biotopes are preserved. These biotopes are listed as follows:

- Willow-poplar forests (*Saliceta albae-fragilis*) along riverbanks and backwaters
- Oak-ash-elm mixed forests (*Querceto-Fraxineto-Ulmeta*)
- Hedges and ditches, previously farm borders, now uncultivated agricultural areas
- Fords, where single old black poplars were left to show travellers and ferrymen the way.

The Law states that, wherever possible, species must be preserved in natural areas. The utilization of natural areas is only authorized in specific, nature-friendly manners. In this respect the plant associations found alongside rivers and biotopes influenced by water bear great significance. For this reason, the status of such plant associations can only be changed with the prior permission of the designated Nature Protection Authority (NPA), for example a National Park (NP) or a Nature Protection Directorate.

#### *In situ* conservation in nature protection (NP) areas

The Law states that wild species, individual representatives of these species, associations of such and their biotopes can be granted protection. Plant associations of black poplar (*P. nigra* populations) can thus be granted protection if this should prove necessary. Forests in NP areas do in any case possess a primary forest protection function. This fact has fundamental influence on all forest management practices and activities, including the choice of silvicultural methods, the term of logging activities, etc. In protected forests, nature-friendly methods must be employed in order to preserve the natural structure and the status of the species and its associations. In NP areas, reforestations can only be carried out with autochthonous species. The ecological conditions are decisive in determining which species can be planted at certain sites. This regulation will without any doubt alter the present overall role of the black poplar. There is likely to be an increasing demand for reproductive material so that black poplars can be used for the afforestations in the NP areas.

The described process can also be expected to influence the present system of procurement of reproductive material. Trees or groups of trees growing in NP areas and not included in the forestry register can only be cut down with the permission of the designated NPAs. The NPAs must be consulted in all cases of forestry management activities. No farming activities must be carried out in NP areas without authorization. The owners will be prosecuted for any unauthorized activities in NP areas. If ownership rights to NP areas are to be transferred, the State has priority for purchasing these rights. No NP areas already in the possession of the Hungarian State can be sold to other owners.



The extracts from the new Law may be of assistance in the development of projects for the *in situ* conservation of endangered species in Hungary. However, the paragraphs only provide the legal framework for the more efficient practical conservation efforts on the black poplar. The actual results will depend on how these possibilities are exploited.

## Belgium

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The status of *Populus nigra* in Belgium has been discussed in the Report of the first meeting of the *P. nigra* Network, Izmit, Turkey, 1994. In the meantime, the following activities have been developed.

### Conservation activities

The main activities focused on the conservation of the existing material in the genebanks. Those genebanks were established some 35 years ago and were managed for adult tree plots. They are now reaching maturity.

Depending on the quality of the material, both cuttings and grafts were used for rejuvenation. More than 80 clones are involved. The material is conserved in the experimental nursery of our Research Institute and at the Walloon Forest Research Station.

This programme will be continued next spring. The material will be used either to install new *ex situ* conservation plots (stool-beds and adult tree plots) or for identification purposes of the clones according to the EUFORGEN descriptor list.

Furthermore, the collection is extended with some remnants of the species found as pollarded trees in the Maas and the Iézer valley.

### Documentation and establishment of the databases

The collection of the EUFORGEN Passport data was started this year, as so far no or few data were available. Information on the *ex situ* collection of the Institute for Forestry and Game Management is checked in the field and missing data are assessed on existing *in situ* material. As suitable material will be available out of grafts and cuttings of the conservation programme, evaluation of the descriptors of nursery plants will be started for the coming years.

### Research

A research programme funded by the Ministry of the Flemish Community started in 1996, which includes a study of the genetic diversity of mainly Belgian *P. nigra* clones, and the study of introgression of *P. euramericana*, using controlled back-crosses. Both isozyme and RAPD techniques will be used.

### Public awareness

A study has been made on the legal possibilities to protect *in situ* material of *P. nigra* in Belgium. As a try-out a proposal was presented to the Flemish Administration responsible for Monuments and Sites, in cooperation with a local authority to protect a pollarded *P. nigra* tree as a monument. In this context also the press was mobilized.

A training session for identification of *P. nigra*, using the EUFORGEN identification sheet, was held at the Institute for Forestry and Game Management for the landscape managers of the Flemish Land Company, which are in charge of the land management.

This activity has resulted in the identification of some *P. nigra* specimens in the Iézer Valley and in their future legal *in situ* protection. The specimens were endangered by re-allotment activities of a large part of the Iézer valley.

General articles on conservation of forest genetic resources in both forestry and nature conservation journals were published and received a good response.

## Croatia

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The activities on conservation of the European black poplar (*Populus nigra* L.) genetic resources in Croatia started in 1993. In 1996, we continued with the selection of trees of the European black poplar in the western part of the Sava river and on the lower part of the Drava river. Another 27 old trees have been selected. In addition to 50 clones of the European black poplar selected in the last two years the total number of cloned trees thus amounts to 77. Selection was made in the regions longitudinally near the Sava, Drava and Mura rivers, the region of eastern Croatia (Sava, Drava, Danube rivers) being still inaccessible for us (UNTAES zone). As a rule, old trees of the European black poplar are present as individual trees, less often as groups of trees.

Table 1 shows the total number of selected and propagated trees of European black poplar and their number included in the clonal archive. In March 1996, we established a clonal archive of poplars in which are included 34 clones. The clonal archive is near the Mura river in the northern part of Croatia and in the spring of 1997 it will be successfully completed with another 43 clones.

**Table 1. Number of selected trees of European black poplar, propagated and included in the clonal archive.**

Age (yrs)	No. of selected <i>Populus nigra</i> trees	
	Propagated	Included in clonal archive
<15	12	12
50	11	4
70-90	44	12
120	2	–
200	8	6
Total	77	34

Selection of good-performing individuals from young forest stands of the European black poplar is also very interesting since in this way it is possible to select new genotypes which will be well adapted to specific, local site conditions in which the silviculture of Eastern cottonwood (*P. deltoides*) and hybrid poplars *P. x euramericana* is problematic.

In accordance with the decision of the Croatian Ministry of Agriculture and Forestry from 19 February 1996, on the lowland forest near the Drava river (Forest range office Slatina), 620.30 ha of natural mixed stands of European black poplar, white poplar and white willow are excluded from the regular forestry management. This decision is in agreement with the *in situ* preservation of genetic resources of natural broadleaved tree species in Croatia.

## France

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A national programme for *Populus nigra* conservation has been recently approved by the National Technical Commission for Forest Genetic Resources Conservation (Ministry of Agriculture). This is the general framework including objectives and practical considerations, while several tasks have already been in progress and specific means still have to be found for the remaining planned tasks.

### Justification and objectives

*Populus nigra* is a particular model for gene resource conservation, as pioneer species of a 'perturbed' ecosystem (Herpka 1986; Zsuffa 1974). A high level of genetic diversity within populations is generally observed: this can be explained by some characteristics of the colonization process through seedling cohorts, with limited redundancy due to vegetative propagation at the adult tree level (Legionnet 1996). Genetic differentiation among regions, or among stands within regions, is very low (Malvolti and Benedettelli 1993; Legionnet and Lefèvre 1996), but not null in particular for non neutral traits (Legionnet 1996), and local adaptations are suspected. Theoretically, the introgression should be detectable (D'Ovidio *et al.* 1991; Faivre Rampant *et al.* 1992a, 1992b), but the process still has to be studied (Legionnet 1996). The taxonomy status of *P. nigra* is currently re-examined on the basis of DNA markers (Smith and Sytsma 1990; Faivre Rampant *et al.* 1995).

Three factors are supposed to contribute to the genetic erosion of *P. nigra*: the diminution of the natural riparian ecosystem, the possible introgression with cultivated varieties (Cagelli and Lefèvre 1996), and a rapid evolution of the parasite populations due to intensive poplar culture (Pinon *et al.* 1995). The first factor has led to almost an extinction of natural stands in some parts of the western margin of the distribution range of this species. The consequences of the two other factors still have to be clarified.

The Ministry of Agriculture has supported research activities related to *P. nigra* conservation since the 1970s (Teissier du Cros 1977; Duval *et al.* 1993; Lefèvre and Legionnet 1994; Lefèvre *et al.* 1994, 1995; Legionnet 1996). One of the practical results is the clone collection currently maintained by INRA and administrative nursery in a stool-bed and a network of plantations (Table 1). The French breeding programme is also concerned with *P. nigra* germplasm (Villar *et al.* 1995). A more general study of the situation of poplar in the landscape was started by CEMAGREF (Le Floch 1995). In the international context, the ongoing pan-European preoccupation about *P. nigra* and the collaboration within the EUFORGEN Network represent a new motivation for the implementation of a national programme.

At the national scale in France (which is situated in the marginal part of the species' distribution range), the conservation of *P. nigra* has two objectives:

- to maintain the various gene pools that founded today's diversity
- to maintain the particular local adaptations.

The gene resource conservation of forest trees needs a long-term budgetary allocation, which is not necessarily linked with the economic interest of the species

considered. Therefore, different strategies should be combined from the beginning. *Ex situ* conservation is efficient in the short term, but it remains static. Dynamic conservation, potentially generating new diversity through recombination and selection, can be achieved either through *in situ* management, in natural conditions, or through breeding populations under recurrent selection: both rely on the long-term approach. The cost of a breeding population has to be justified by the expected genetic gain of the new varieties. It seems more difficult to justify important financial support for *in situ* conservation; therefore this approach will rely mainly on the existing protected areas. Safe guidelines may be given for some aspects of *ex situ*, *in situ* or dynamic conservation, and research is still needed if we want to optimize the integrated conservation strategy.

**Table 1. Clone collection of *Populus nigra* (accessions available in 1996).**

	France	Foreign	Misc.	Total
Clones	347	59	3	409
Progenies (clones)	75 (772)	0	0	75 (772)
Controlled crosses	—	—	18	18
Total sites or countries	117	6	—	—

### Resource inventory and characterization

We lack basic information on the extent and spatial distribution of the remaining resource. This is partly because *P. nigra* is not individualized in the various forest inventories. We give the following objectives to the resource inventory:

- describe the present situation, which would allow a follow-up
- identify the drainage areas where the species has retreated
- identify the drainage areas where important reservoirs of the species remain
- characterize the species distribution (isolated trees, stands)
- provide guidelines for completing the clone collection.

A first inventory concerns remarkable trees (age and/or dimensions), either isolated or within natural stands. Priority will be given to the regions which are not represented yet in the clone collection, or where the risk of natural introgression is supposed to be high. The identification sheet from EUFORGEN will help to get information from various sources (natural reserves, ONF, associations); these data will be centralized and entered in a computerized database which has to be compatible with EUFORGEN recommendations (and will form a basis of those at the same time).

Then, *P. nigra* stands should be localized and characterized (for black poplar, a stand can be defined as a group of adult trees from both sexes, producing seeds which are able to be regenerated in a suitable area). A typology of the stands is expected from the EUFORGEN Network as well. Preliminary information is available in the aerial pictures used by the National Forest Inventory. A second inventory 5 years later would give useful information about the possible evolution of the resource; then the interval between inventories should be adjusted. Information about remarkable trees might be recorded, and will have to be computerized as well.

A regular survey of the pathogen population in the native stands, and in the network of populeta is proposed. This in fact has already started (INRA and CEMAGREF).

**Ex situ conservation: clone collection**

A collection of clones with the best possible representation of the existing diversity will be conserved. Collecting stem cuttings rather than seeds is proposed in order to simplify the collection, to limit genetic redundancy, and to prevent screening introgressive forms in the progenies.

The existing collection does not fulfil the two objectives previously mentioned, and it has to be completed. Today, we have no precise idea of the founder genepools; therefore the completion of the collection will focus on the objective of representing the various local adaptations, and sampling strategy will be stratified according to the geographic origin. Given the particular distribution of the species, with many small groups of trees and few extended stands, we propose to weight the representation of each collecting site according to the logarithm of the number of flowering trees rather than the effective number (Brown 1989). Finally the strategy will be as follows:

- gather an initial collection of 1000 clones from the whole country: all will be maintained as adult tree plots, but only 500 of them, representing all the collecting sites, will be evaluated and maintained in stool-beds (active collection)
- for each collecting site, the maximum number of clones in the collection is determined according to Table 2
- the set of 500 clones for the active collection will be regularly updated, e.g. to incorporate particular phenotypes or to adjust the representation of certain collecting sites.

**Table 2. Representation of the collecting sites in the initial collection.**

No. flowering trees in the stand	Maximum no. of clones in the collection
<10	1
10 – 20	2
20 – 50	3
50 – 150	4
>150	5

In a second phase, when more information is available on the founder genepools (in particular based on cytoplasmic DNA markers), zones of particular interest might be identified and new introductions necessary to complete the initial collection.

The evaluation of the active collection, in nursery or laboratory, will follow the joint EUFORGEN guidelines:

- adaptive traits of moderate to high heritability (phenology, resistance)
- morphophysiological descriptors, and possible variants
- simple biochemical markers (isozymes)
- fine molecular characterization (marker of introgression).

The *ex situ* conservation network will consist of a stool-bed of 500 clones, field plots containing all the 1000 clones, and any plantation trial linked with the poplar breeding programme. The effective number of ramets per clone will also follow EUFORGEN recommendations: 4 ramets in stool-bed, and 3 adult trees at a density of 200-400 trees/ha (8-15 ha for the 1000 clones). A stool-bed has to be renewed every 7 years, adult plots every 20 years. One can also think of cryoconservation since black poplar is probably one of the forest tree species for which the technique could be used.

***In situ* conservation**

The strategy would be first to benefit from existing protected areas which could include the conservation of *P. nigra* in their management objectives, and, second, to pay special attention to the regions of species retreat. *In situ* conservation of *P. nigra* is considered in the framework of the management of riparian ecosystem and differs from other *in situ* programmes in forestry.

Various types of protected areas might be involved in that programme, depending on their longevity and their management rules. The French Natural Reserves (Ministry of Environment) would be the main operational structure: the inventory and characterization of the *P. nigra* resource is achievable, and part of the information is already available. For example, these Reserves have settled 300 experimental plots in the riverside forests, where 10 000 trees are individually observed (Pont 1994, 1995). Moreover, the management of these areas has been considered (Michelot 1995). There are other protected and managed areas (life programme, national or regional programmes) whose participation is required at least for the inventory task, but only perennial programmes could be involved in the *in situ* conservation network. This second type of protected area involves regional institutions and ONF. Finally, many other protected areas should not be included in the conservation network, generally because of the lack of management rules.

In the drainage areas where the species has become rare owing to human activity, some 'relic' groups of flowering trees will be identified and protected, as a potential basis for new colonization. The nature of the protection still has to be defined. It is also possible to plant material of strictly local origin. Recommendations have to be given for favouring the re-establishment of poplar seedlings in the case of newly set-aside riverbanks.

**Dynamic management**

In 1995, INRA started a study on the best strategy for managing breeding populations of black and balsam poplars. In the breeding programme, *P. nigra* is only involved as a parent species for euramerican hybrid varieties. The recurrent selection strategy for that species is mainly aimed at maintaining a large genepool, and relationship is controlled through the follow-up of genealogies. Each family at generation (n) contributes to the next generation: clonal selection for the generation (n+1) essentially occurs within family. This strategy will start with a first set of 50-60 genitors. The selection is made in two steps: first in the nursery for juvenile growth, resistance and wood specific gravity, then a stratification of the selected clones, based on fine components of the complex traits and phenology, will be used to identify the parents for interspecific hybridization.

Another approach would be to set up a network of artificial plantations in the wild: the scientific basis for such an operation has to be completed, and it is considered as purely experimental. Adult tree plots would be installed in various contrasted conditions, at wide spacing (for better flowering). Silviculture will be reduced to the first 2-3 years, then adaptation to the environment will govern pollen and seed set of the trees. In the longer term, seeds could be collected in these artificial populations for a new generation (according to available means at that time). These populations should be maintained for at least 15 years. Costs are reduced to the first years. This is an opportunistic approach which could be integrated in the projects of re-establishment of riparian forests (various institutions involved). These plots can also be used for scientific studies, e.g. mating system, geneflow with local resources.

### Research

The first operations of the programme may start now. The level of precision required for inventory and characterization of the resource under *in situ* conservation, as well as the stand descriptors, has to be clarified. For the rest, research needs in the short term correspond to the EUFORGEN Network recommendations:

1. distribution of founder genepools (DNA markers with maternal inheritance)
2. importance and consequences of the introgression process
3. stand dynamics and geneflow, in relation to the global ecosystem
4. risk assessment, modeling and experimental validation.

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## Spain

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Since last year the state of *Populus nigra* conservation has not changed significantly. We have been maintaining the collections *ex situ* and prospecting new areas.

### **Conservation *ex situ***

The collection at SIA-DGA (Zaragoza) is maintained as adult trees (arboretum): more than 110 clones from Ebro Valley. Forty-two clones from this collection were established in stool-beds (1 year old).

### **Prospecting**

Twenty-three new clones have been collected from natural stands in the Ebro and Duero valleys. This collection is maintained in a greenhouse at CIFOR-INIA (Madrid).

### **Conservation *in situ***

*Populus nigra* in Navarra (Ebro Basin) is growing in Protected Areas. These stands are under protection for the conservation of the wildlife, so they have no management.

In the Duero Valley, *P. nigra* stands are not protected and they are actually menaced.

In order to obtain financial support to study natural populations from Ebro Valley and SIA and CIFOR collections, a joint proposal (SIA-DGA and CIFOR-INIA) was presented to the National Programme for Investigation and Development.

## Conservation of genetic resources of *Populus nigra* in the Czech Republic

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### Present distribution and reasons for reduction

In the Czech Republic the native black poplar (*Populus nigra* L.) is an endangered species. The original sites of black poplar are found in conditions along streams in inundation areas, from lowlands to uplands. From the original regional black poplar populations only isolated old trees or small groups have remained. Large parts of the landscapes are without this species.

The loss of original sites by large landscape drainage and regulation of streams makes natural regeneration of this species almost impossible. Black poplar was displaced by the cultivation of fast-growing *P. x euramericana* cultivars. Spontaneous interspecific hybridization of black poplar with imported cultivars (*Populus nigra* L. var. *italica*, *Populus x euramericana*) threatens the genepool of the native woody plant in those few cases when its natural regeneration occurs (Mottl and Dubský 1995).

The vegetative propagation of old trees of *P. nigra* is problematic from the physiological point of view. A lot of old trees most likely originate from the vegetative propagation of cut-down trees. The lack of success in experiments in cultivation of vegetatively propagated old trees of *P. nigra* can be explained not only by the drainage of landscapes and the spread of diseases during the cultivation of hybrid poplars, but also by the old age of these clones.

The conservation of our *P. nigra* genetic resources is possible by new generatively propagated populations obtained from the controlled crossing of 'select' trees. Their plantation in various sites, from optimum to extreme, will enlarge the genetic diversity.

### History

Conservation activities of the genetic resources of *P. nigra* began in the 1950s in the Research Institute of Forestry - Research Station Uherské Hradiště with prospecting and collecting of 'select' trees (trees selected according to the conservation of genepool and their production properties). Controlled intraspecific crossing of these trees was made and produced a large number of seedlings. These seedlings were planted in the central row of windbreaks in South Moravia. Because of the previous bad experiences with the cultivation of vegetatively propagated *P. nigra* old trees, no interest was shown in using these plants in forest planting.

These experiences were confirmed in 14 trial plantations with various poplar clones established from 1953-67. Fifty-six clones of *P. nigra* were planted and positive results with several clones were obtained only at the optimum sites, which were sometimes flooded.

By 1985, 78 select trees of *P. nigra* were collected in the clone archives in the Research Station Uherské Hradiště.

## Present situation

### Select trees

In 1985 more extensive research on the conservation of genetic resources of *P. nigra* started with prospecting other select trees and the evaluation of black poplars in the new windbreaks.

In 1987 the Research Institute of Ornamental Gardening joined the programme with several research projects (from the Ministry of Environment, the Grant Agency of the Czech Republic and the Ministry of Agriculture). The work concentrated on three areas: windbreaks with *P. nigra* in South Moravia, the central Moravia Basin and the central Labe Basin. In these localities new select trees were prospected and detailed characteristics of their habitus (based on 17 criteria) were given. The trees are ready for incorporation into the Central records in the Research Station Uherské Hradiště. Since 1987, 204 select trees have been marked in the field, described and put in the stand maps.

In 1993 the investigation of the windbreaks planted with *P. nigra* seedlings in the 1950s was finished. The total length of the windbreaks is 15.8 km, parts with *P. nigra* were registered in the length of 10 km. In particular parts of windbreaks the silvicultural measures were suggested to protect trees of *P. nigra*. Only a small part of the windbreaks is situated along streams. Most windbreaks were planted in an agricultural landscape with a lower level of groundwater (*ex situ*). In these sites *P. nigra* propagated by seed grows better than *P. x euramericana* cultivars. In the windbreaks, 97 select trees (46 f, 51 m) were chosen, of which 32 trees were vegetatively propagated and collected in the clone archives (Dubský and Mottl 1993). Five other clones were collected in the 1960s. It will be possible to use these clones with certain properties in programmes of reintroduction of *P. nigra* into the landscape, where it is not possible to use progenies propagated by seed, or in afforestations.

In the central Morava Basin (the Litovelské Pomoraví Nature Preserve, the Reserve of the floodplain forest Zástudáncí, the Bystrice Valley) 50 select trees (19 f, 31 m) were chosen, of which 14 were vegetatively propagated to the clone archives (Mottl and Dubský 1996). Besides these, other high-quality black poplars were found in the Svratka and Jihlava Valleys, in the Reserve Mlýnsko and in the urban vegetation of Brno.

In the central Labe Basin (small reserved areas, urban vegetation of Podebrady and Brandýs) 42 select trees (19 f, 23 m) were chosen, of which 15 were vegetatively propagated to the clone archives. In the Vltava Basin (urban vegetation of Prague, streamside stands) there are 15 other select trees (8 f, 7 m), of which 4 are in the clone archive.

Other quality trees were found in the Ohře and Bílina Valleys in southwestern Bohemia. In 1996 a country-wide investigation of extreme stands of *P. nigra* (uplands with altitude 400-750 m) was made and 18 black poplars (11 f, 7 m) were identified. Next year inventories of *P. nigra* distribution will be made in other parts of the Labe Basin, in South Moravian valleys and in the Moravian Carpathians.

Besides vegetative propagation and collection into archival stool-beds, the select trees can be used for controlled interspecific or intraspecific crossings.

By 1996, 160 clones of *P. nigra* from the Czech and Slovak Republics had been collected in the clone archives in the Research Station Uherské Hradiště. This Research Station takes part in the creation of an international clone archive, which was established in Casale Monferrato in Italy.

**Ex situ and in situ plantations**

In 1989 an adult tree plot (with land area of 9.6 ha) containing a collection of poplar clones suitable for plantation into landscape was established in Prùhonice (*ex situ*). On the land area of 2.6 ha, 27 clones of *P. nigra* from stool-beds (collected in the 1960s) were set out. The growth of 7-year-old black poplars is now very good and the differences in tree height, habitus and foliage of these clones are already perceptible.

In 1994, 7 adult tree plots (with a total area of 4.7 ha) with 2-year-old seedlings of *P. nigra* were planted in the Litovelské Pomoraví Nature Preserve (*in situ*). Nine progenies from controlled crossings in greenhouse conditions and three progenies from open-pollination of three female trees used in the crossings were set out. As parent trees, select trees from all over the Czech Republic (windbreaks in South Moravia, Labe and Vltava Valleys) were used. Four plots with 1368 planted trees are evaluated each year.

In the near future we plan to plant two *in situ* plantations with 2-year-old seedlings, one in 1997 which will be a small streambank stand (about 0.8 ha) in the Litovelské Pomoraví Nature Preserve with 8 progenies (about 250 seedlings) and a second in 1998 will be several forest plantations (about 10 ha) in small reserved areas in the Labe Valley with 13 progenies (about 4000 seedlings). Seedlings from the controlled crossings of parent trees from each regional population of *P. nigra* will be planted together with vegetatively propagated transplants of parent trees.

It is planned to make additional controlled crossings in order to conserve regional populations (e.g. in the Moravia Valley, in uplands areas – the Ostravice Valley) and also to hybridize select trees from various areas to gain clones with good yield properties. Plantations of seedlings from these crossings can be used for genetic analysis of parent trees and, after 20 years, for the selection of new clones and select trees. Select trees of *P. nigra* will be also used for interspecific crossings with *P. deltoides*.

**Isoenzyme analysis of genus *Populus***

To distinguish hybrids *P. x euramericana* from species *P. nigra* we use isoenzyme analysis, horizontal starch-gel electrophoresis. This method was used for species *P. trichocarpa* (Weber and Stettler 1981) and for species *P. deltoides*, *P. x euramericana* and *P. nigra* (Rajora 1989). Our results are partially different from the results described by Rajora. From applied enzyme systems (PER, IDH, MDH, PGI, 6-PGD, LAP, ACO) the best results were received with 6-PGD and LAP, which make possible a reliable and easy identification of hybrids *P. x euramericana*.

It is possible to distinguish hybrids *P. x euramericana* from *P. nigra* according to morphological traits. That is why we concentrated on introgressive hybrids *P. nigra* x *P. x euramericana*, which can arise spontaneously and their identification is much more difficult. For this purpose we made several crossings. An isoenzyme analysis of 40 offspring from the crossing of *P. x euramericana* Marilandica x *P. nigra* (select tree 880044) was made. In both enzyme systems 6-PGD and LAP the segregation of both parent phenotypes in ratio 1:1 occurred. When using both systems together there is about 75% probability of identifying hybrids. These two enzyme systems do not distinguish *P. x berolinensis* and *P. trichocarpa* from *P. nigra*.

We are going to use this information among others in the evaluation of progenies in sites with natural regeneration of poplars. The genetic analysis is mainly used for the evaluation of select trees of *P. nigra* included in the clone archives and used for crossings. By this method it is intended to also characterize regional populations of *P. nigra* in the Czech Republic.

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## ***Populus nigra* in Austria: rare, endangered, not recognized ?**

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### **The situation**

#### ***Floodplains in Austria - natural *P. nigra* habitats and their deterioration***

In late 1984, conservationists prevented the construction of a hydroelectric power station in the middle of what is believed to be the last vast natural floodplain forest in Austria, along the Danube at Hainburg between Vienna and the Hungarian border. This fierce confrontation, which led to some violent scenes when the first (and last) trees were felled, had a tremendous impact on the public perception of floodplain forest conservation. It also helped to boost research funding in such areas – for a few years. Austria joined the Ramsar Convention (protection of waterfowl habitats) in 1983; 3 of the 4 areas included in this treaty have relevance to *Populus nigra* conservation (Danube/March/Thaya floodplains, Untere Lobau, Rheindelta/ Bodensee). The current construction of another hydroelectric station in Vienna is accompanied by a big public relations campaign. Nevertheless, emphasis in floodplain conservation efforts is first on water, then on forest ecosystems, and within them, on animal species. While hybrid poplars planted in blocks are widely seen as 'unnatural', *P. nigra* and its genetic problems are neglected by the general public and the major conservationist groups.

Lazowski (1989) has summarized the occurrence of "Auen" (floodplains) in Austria (Fig. 1). Continuous major floodplain forests are still found along the Danube and March rivers (between Vienna and Hungarian/Slovak border), the Leitha and the Mur river at the Slovenian border. Altogether, floodplain forests comprise 1.1% of Austria's forests (426.6 km<sup>2</sup>, calculated from Schadauer 1994). Floodplain forest soil types cover 700 km<sup>2</sup> (Kilian 1995). The biggest share of these areas is situated along the Danube river (300 km<sup>2</sup>), especially in the Lower Austrian part (230 km<sup>2</sup>, Ruhm 1990).

Black poplars (including hybrids) comprise only an insignificant share of Austria's forests in terms of area and individual trees. Distribution maps for *P. nigra* exist only for the Federal States of Salzburg (Wittmann *et al.* 1987) and Kärnten (Hartl *et al.* 1992), but differential identification of *P. nigra* vs. hybrids is not always beyond doubt. Kärnten is the only Federal State that has put *P. nigra* on the Red List (level 5; Hartl *et al.* 1992). Botanical data indicate an occurrence of *P. nigra* in all Federal States (Fischer 1994); however, this includes ornamentals, windbreaks and single trees in parks, etc. A more exact estimate of the potential present distribution may be the map of (lowland) floodplains in Fig. 1. Almost certainly, no *P. nigra* stands are left outside of the remaining major floodplain areas, but single trees may be more widespread. The Kärnten distribution map is remarkable as only very small patches of floodplain forests are left there, but *P. nigra* is indicated in what may be taken as its potential former distribution range, the lower river valleys. The only suitable region for *P. nigra* in Salzburg, a mountainous federal state, is the Salzach valley below the city of Salzburg. The upper river valleys in the Alps, as well as areas of higher elevation in the north of Austria (Mühl- and Waldviertel) are generally considered outside of the distribution range of *P. nigra*. Data from neighbouring Bavaria (Gulder 1996) confirm that the species reaches its vertical limits at an elevation of approximately 400 m in this climate zone.

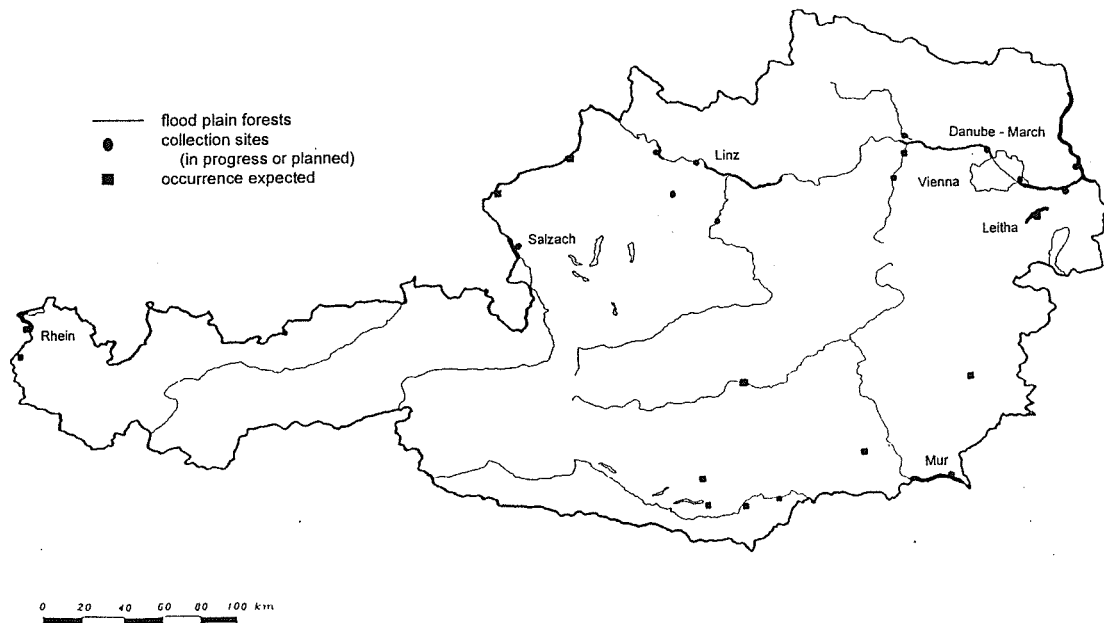


Fig. 1. Floodplains in Austria.

#### ***The Danube/March river systems***

Floodplain forests have always been close to human settlements; their intensive use is a consequence. In Austria, hunting has had (and still has) a major impact on these forests. Timber production took off only in the second half of the previous century, when fuel wood consumption declined. Hybrid poplars came in and replaced *P. nigra* on many suitable sites. A report from the beginning of this century claims that *P. nigra* has been deliberately removed from a Danube floodplain forest because of its poor wood quality (unsellable fuel wood; Wendelberger-Zelinka 1952a). The main hybrid cultivars have been successively Marilandica (the Danube poplar, Jelem 1974) and Robusta, followed by the Italian clone I-214 and others. Reliable dated reports of hybrid introduction are 1860 for Zögersdorf/Stockerau (Marilandica, after Jelem 1974) and 1907 for the Wallsee area (Wendelberger-Zelinka 1952a).

Jelem (1974), in his description of the Danube floodplain forest sites, reports that *P. nigra* was still common in the Eferding Becken in the early 1960s (where today only a few trees can be found; F. Sternberger, pers. comm.), the region of Klosterneuburg (especially Korneuburg region) and the Untere Lobau. Of these, Untere Lobau is a region where many *P. nigra* trees are still found. Soil and site conditions along the Danube in Lower Austria were mapped by colleagues in our Research Centre in the late 1980s (Mader/Margl Standortskarten). Of these, the areas of Wolfsthal, Mannswörth, Hainburg, Stockerau and upriver towards Tulln have only few potential *P. nigra* growing sites, while there are patches near Neuaigen, Langenschönbichl, Utzenlaa and Frauendorf/Winkl (opposite Zwentendorf) that are generally richer in *P. nigra*-compatible soil types. Ruhm (1990) compared actual and potential vegetation in this latter area: most of the sites suitable for *P. nigra* have been heavily planted with hybrids and other tree species.

Hydroelectric power stations have been built covering almost completely the stretch of the Danube in Austria: approximately 250 km of 350 km are banked up (Donaukraft information leaflet, no date). These power stations caused major



changes to the landscape. In the remaining areas, flood dams prevent 'natural' floods except for sites very close to the river. About 110 km<sup>2</sup> of floodplain forest are situated next to stretches of the river free from the direct impact of power stations (Lazowski 1989). Absence of floods allows intensive agricultural use of the fertile soils. The sudden drop in the groundwater table after such building measures leaves older trees without sufficient water supply. In the long run, poplar stands are succeeded by hardwood forests. The absence of regular floods also prevents favourable germination conditions for poplar seeds on newly exposed open soil and gravel. On the other hand, dense poplar regeneration is often observed on soil disturbed by building activities. Only very recent power stations along the Danube are providing 'water donation canals' for the forests.

A special feature of floodplains upriver, but not too far west, are so-called 'Heißländs' (meaning 'hot embankments'). These are sites with gravel almost up to the topsoil formed by strong floods. Because of low water retention capacity, these sites are very dry and carry a shrubby or meadow vegetation. *Populus nigra* is the only autochthonous tree species that can endure such conditions. Heißländs are common around Tulln, but are rare from Vienna downriver. One exception is the Untere Lobau in Vienna where 20% of the total area are Heißländs with *P. nigra* trees or stumps of poor growth.

Western (upriver) floodplains of the Danube have a greater share of 'soft' soil types more suitable, in general, for poplars and willows. Wendelberger-Zelinka (1952b) reports of a former practice to harvest black poplar for fuel wood every 2 to 5 years (similar to present-day 'energy forests').

Along the March/Thaya rivers, *P. nigra* is rare (Jelem 1975). Regulation buildings reduced suitable sites to a narrow band along the river edge. The lower March floodplains (Slovakian border) have harboured a WWF nature reserve since 1970. There are not many *P. nigra* trees left; however, the influence of planted hybrids is also not very strong (P. Ebner, WWF Austria, pers. comm.).

#### **Other rivers**

**Mur:** H. Otto (Amt der Stmk. Lreg., pers. comm.) reports on the occurrence of *P. nigra* stands near Bad Radkersburg in southwestern Styria (Slovenian border). Gravel pitches are the main problem for conservationists; some of them have been converted to recreational lakes. The land is owned by many small farmers who do not plant hybrids very frequently. Upriver, a single specimen can be found (Unzmarkt - Puxer Au, elevation approx. 750 m!). Near Judenburg (Thalheim), hybrid plantings have replaced native *P. nigra* because of a nearby paper factory.

**Rhein:** The nature reserve of the Rhine Delta (Lake Constance) comprises 1270 ha (Gepp 1985). Single black poplars are left there; management plans suggest selective felling of hybrid poplars (DI Albrecht, Vlb. Lreg., pers. comm.).

**Eastern Austria:** The Leitha river is an example of black elder/common ash floodplain forests with less *P. nigra* (Lazowski 1986). Remnant floodplains occur along the Schwechat river near Traiskirchen, the Lafnitz river (Styria/Burgenland border), and very small remnants along the Raab shortly before the Hungarian border. All these areas are home to single individuals of *P. nigra*, as are the Kainach and the Sulm rivers in Styria (Otto, pers. comm.), and the Ybbs, Erlauf and Traisen rivers of Lower Austria.

**Western Austria:** Thirty-five km<sup>2</sup> of floodplain forests are situated north of Salzburg (Salzach river). There are further patches of floodplain forests along the Inn, bordering Bavaria (Gulder 1996). Remnants of floodplains are found along the Traun in Upper Austria where gravel production is strong.

Smaller rivers and brooks in agricultural land sometimes still support a narrow band of trees along their edges, where *P. nigra* trees can be found. A problem in this setting is that *euramericana* hybrids are also widely planted as windbreaks between fields, so that introgression becomes possible. Castles and large country homes in the east of Austria are often situated in former floodplain forest areas; sometimes their parks and gardens are still home to remnant original vegetation including trees (e.g. Laxenburg south of Vienna; Wendelberger-Zelinka 1960).

There is currently no effort being made to conduct a comprehensive census or inventory of the species. Apart from a few stands in the Lobau, no *P. nigra* forests are known to us as yet. Single individuals, however, are quite common, but some uncertainty about introgression remains with these.

All sorts of hybrids are often planted as ornamental trees. The variety *italica* is virtually ubiquitous, found even in some mountain valleys. Alleys of *italica* poplars are very popular, and another site where it is almost always found is the football ground of each village and town.

### **Main current problems**

#### ***Identification of backcrosses and pure P. nigra***

The more generations of backcrosses have occurred, the more difficult morphological identification becomes. We also find it hard to identify F<sub>2</sub> plants beyond doubt. This applies especially to young plants. We are therefore investigating molecular genetic techniques for distinguishing the species and hybrids. We are using published DNA markers (Bradshaw *et al.* 1994) and chloroplast DNA intergenic spacers, both with the PCR technique. So far, we were successful in identifying a *P. nigra* allele for marker *win3*, a wound-inducible gene. The *P. deltoides* allele at this locus is different from *P. nigra*, *P. trichocarpa* and other balsam poplars. This marker does not allow the assessment of single individuals beyond the first generation of hybridization, but if applied to a whole population (e.g. seed or plants collected from a site) it is possible to estimate the percentage of hybrid poplar alleles in the plant material as a whole.

#### ***Regeneration and establishment through seed***

We sometimes observe seedling establishment on disturbed sites, mainly after building work, but almost none in natural habitats because of the absence of floods.

#### ***Introgression and remaining genetic variation***

Early reports (Wendelberger-Zelinka 1952a, p. 77) held that introduced hybrids, being mainly male, would not be able to regenerate through seed (this belief is in contradiction to the widespread use, at that time, of the Marilandica clone which is female). Nevertheless, planted hybrids do produce seed; there is no reason to believe that they do not produce pollen as well. All combinations of crosses are possible in the laboratory after the F<sub>1</sub> generation (M. Villar, INRA Orléans, pers. comm.) – do they also occur under natural conditions?

We also lack information on genetic variation in the remaining stands and individuals. As a first hint, we observed both male and female flowering in the Lobau with its many *P. nigra* trees.

### Conservation activities

Conservation legislation is a matter of the Federal States, and therefore not uniform. A National Park is planned between Vienna and the Hungarian/Slovak border. The legal base of such a park would be a contract between the administrative authorities and landowners. This park would have great relevance to *P. nigra* conservation. Proposals for management plans consider a gradual eradication of hybrid trees and stands. However, this would give the hybrids a chance to pass on their genes. On the other hand, instantaneous removal of all hybrids would result in large clearcuts, which are also unwanted. A way out of this dilemma may be to use genetic markers to check regeneration sites of black poplars.

The next level of protection is Natur- or Landschaftsschutzgebiete, which are the responsibility of the Federal States. Forestry may be practised in some of these areas, depending on the particular legislation of the Federal States. Larger protected areas with relevance to *P. nigra* are situated at the Rhine Delta and the lower Mur river.

Local authorities in Vienna have declared single old trees to Nature Monuments. These include 17 single *P. nigra* and unspecified numbers along the Alte Donau, the former main river-bed, and at Toter Grund (lists in German on the Internet at address <http://www.magwien.gv.at/ma22/nd/ndindex.htm>) on the flood-prevention dam Donauinsel, which is home to many younger *P. nigra* trees lining both embankments. The protected trees sometimes have a considerable age. We are interested in finding out how many clones these trees represent. Together with the areas of the Praterauen, the Lobau (see below) and the Auparks at Jedlese and Floridsdorf, this makes Vienna a centre for black poplar conservation in Austria (Fig. 2).

In the Untere Lobau region, two stands were indicated to us by foresters, each comprising between 30/40 and maybe even 100 individuals. The larger of those is 130-140 years old, at a site that is now well inland, but used to be an island in the river when the stand was established. Sex distribution seems to be fairly equal, and seed is produced. The other stand is about 60 years old, also naturally established (as judged from the species mix with *P. alba*). Besides these two stands, there are numerous *P. nigra* trees of poor growth and form on dry sites in this area; the age of such trees cannot be estimated from their sizes. There are also hybrid stands closer to the river, inside of a flood prevention dam. Occasional rejuvenation can be observed on river banks (gravel) or from root sprouting.

### Collection of cuttings

The Upper Austrian Forest Nurseries started collecting cuttings in the spring of 1996. Around 20 clones have been obtained (W. Stöckl, pers. comm.). Our own institute has at the same time started a limited collection in the Vienna area which shall be intensified. We plan to establish a reference collection of  $\pm 100$  clones for Austria, trying to find local partners throughout the country that maintain denser collections of local clones.

Current seed and plant trade regulations do not allow the use of vegetatively propagated poplar material for forestry purposes without testing for superior growth performance.

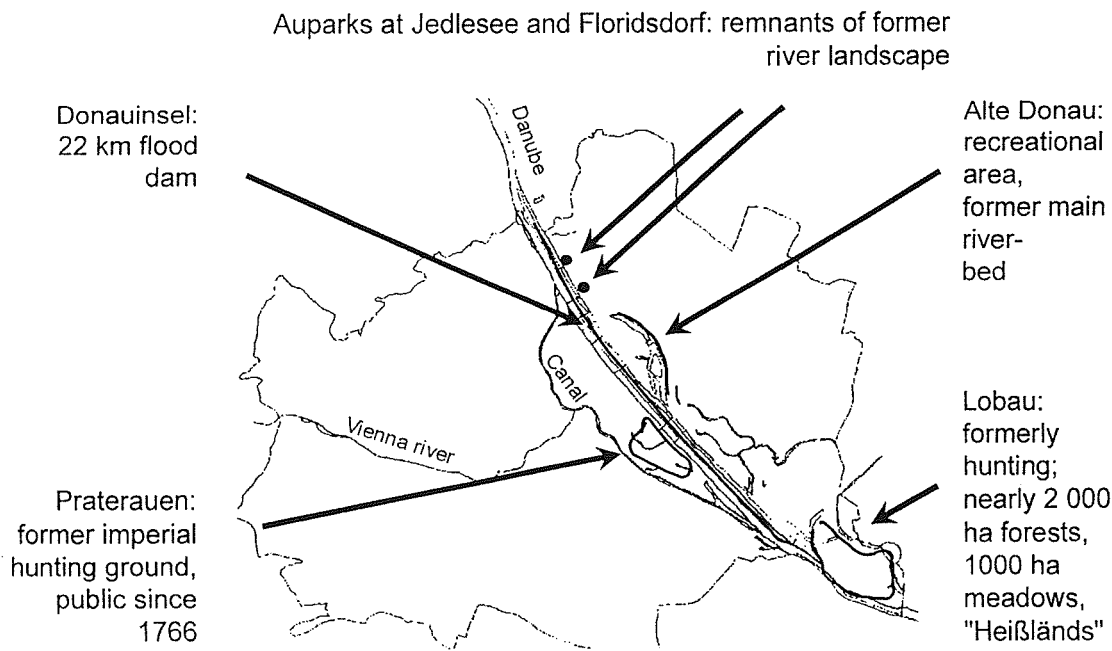


Fig. 2. Occurrence of *Populus nigra* in Vienna.

#### Seed collection

We are also experimenting with seed collected in different areas because this is a dynamic approach and because cuttings from old trees are difficult to root. We obtained several hundred seedlings in 1995 and 1996. The seedlings will be tested for *P. deltoides* alleles in our laboratory as outlined above. Before re-introducing "certified" *P. nigra* plants from seed, we would also like to test for relationship to var. *italica*, maybe with "genetic fingerprints" or microsatellites if such become available.

#### The future

We are concerned about the management of protected areas as mentioned above, having in mind that natural establishment of seedlings is difficult anyway in flood-free areas. Equally important to us is the situation in unprotected areas. Forestry enterprises as well as farms are under enormous economic pressure, and conservation costs money that governments tend not to possess these days. We hope that a future National Park at the Danube may become a nucleus for further conservation activities.

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## Preservation and reproduction of black poplar in Slovakia

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Of the softwood broadleaves, black poplar (*Populus nigra*) represents an important stabilizing element in forests on lowlands and uplands. In consequence of anthropogenic load the area of this species has considerably declined, so that it covers only 350 ha. It is necessary to stress the fact that black poplar does not form continuous stands but it usually occurs in the form of groups and individuals within the forest type: *Querceto-Fraxinetum*, *Ulmeto-Fraxinetum carpineum* and *Fraxinetum-Alnetum*.

In the early 1960s the programme for preservation of autochthonous black poplars was started in Slovakia. The basic task was to determine suitable morphological criteria, since spontaneous hybrids, which were in some cases hardly identifiable, occurred between autochthonous black and introduced american poplars. Model sample trees (buds, branches and leaves) and photodocumentation obtained from the region of upper Orava were chosen to be an objective reference. In that region, american clones of poplar do not occur. The occurrence of the parasite *Viscum album* L. is considered to be the most objective determining indicator of domestic black poplar. It follows from extensive research (during the years 1995-96) that mistletoe may occur only on autochthonous black poplars which grow on extreme sites, such as in pit-run gravel without contact with groundwater. *Viscum album* was registered on all regionalized and prospective clones of *euramericana* hybrids which were older than 20 years. A correlation between site and an occurrence of the parasite has not been confirmed.

On the basis of phenotypic characters, 196 plus (sample) trees were selected for *in situ* preservation. The most valuable individuals were on alluvial soils of the Dunaj and Morava rivers, where some of them reach a height of 39 m. According to dominant phenotypic characters, the 196 plus trees were divided into two groups: **shapable** and **production** trees. Shapable types are characterized by a straight cylindrical stem, with narrow fan-shaped to pyramidal crown, with branches rather thin, set at a sharp angle ( $\leq 45^\circ$ ). A natural branching out of individuals occurs in the canopy.

Production types have in the lower part a straight, sometimes moderately corrugated full-boled stem; at approximately half the tree height, it changes to become a more spreading fan-shaped, semi-spherical crown. From all plus trees of *P. nigra* the reproduction material was obtained which underwent clonal tests in a central poplar nursery as well as on the permanent sample plots. Five clonal archives were established from clones which do not suffer from fungal diseases and pests and which formed a straight stem. Of all tested clones only the 'Baka' clone was equalled in an intensity of growth to the standard 'Robusta'. Because this clone also met other breeding criteria, from 1 January 1996 it has been included in the register of certified poplar clones in Slovakia. An important role in the protection and preservation of domestic black poplars by *in situ* conservation is played by fragments of gene bases – a set of stands represented by original tree species among which a continuous exchange of alleles still occurs. Populations are of a regional character. An area of gene bases varies from 5 to 25 ha and a total area of 153 ha of gene bases had been marked on the territory of Slovakia by 31 December 1995.

In the stands of black poplars with high phenotypic value we have proceeded

with preservation *in situ* and reproduction by a generative method. In the places with stand density of about 0.6 as well as on margins of stands, grass detritus was mechanically removed to protect against seed invasion. The size of partial areas (established plots) was 1 x 1 m and they were laid out in a chessboard pattern. The areas were weeded three times during a growing season and were chemically treated against fungus of the *Fusicladium* genus. Control areas remained without intervention. At the same time the results were compared with sowing in poplar nurseries in the Research Station in Gabčíkovo. The results obtained, shown in the following table, were from September 1996.

Assessed quantitative and qualitative parameters	Free areas		Areas in the poplar nursery	
	Areas mechanically and chemically treated	Control area	Areas mechanically and chemically treated	Control area
Number of single trees/1 m <sup>2</sup>	15	0	79	0
Average height (cm)	11	0	68	0
Health condition <sup>†</sup>	3	0	1	0

<sup>†</sup> Health assessed on a 0 to 4 relative scale, where 0 = undamaged and 4 = severely damaged.

Based on the analysis of quantitative and qualitative parameters of 1-year-old seedlings, it follows that under the assumption of keeping the technological discipline, it is possible in natural conditions to reach good regeneration of black poplar. However, the quantitative parameters are minimally 5 times lower than for generative progeny in stool-beds in the poplar nursery.

*Ex situ* rescue is also being supported by the generative method, as well as by the autovegetative method (poplar parent trees, clonal archives). From the plus trees that are isolated from *Populus deltoides*, or *Populus x euramericana*, seed was obtained and outplanted into the cold stool-bed. At the end of the growing season the 1-year-old seedlings were sized and 10% of the tallest seedlings and seedlings with the highest quality were transplanted.

The greatest height reached by seedlings at the end of the growing season was 167 cm. The percentage of seedling distribution according to height classes was:

Height classes (cm)				
1-40	41-80	81-120	121-160	+160
31%	49%	11%	7%	2%

The selected 1-year-old seedlings were transplanted (spacing: 80 x 25 cm). During the second year the health condition was evaluated regularly and at the end of the growing season the selection was repeated. Twenty percent of seedlings which reached the greatest height showed good resistance, creating a straight stem, and were outplanted in the permanent research plots. The oldest plantings are now 5 years old. Preliminary evaluation revealed that the analyzed populations have a mild or moderately heavy crooked stem with a considerable number of shoots in the lower part of the stem, a spherical crown and branches set at an obtuse angle. With the exception of some individuals, they are regularly attacked by fungal diseases and pests. In comparison with a standard poplar 'Robusta', they reach smaller growth parameters by 12%.

**Conclusion**

In consequence of continual anthropogenic load, the area of domestic black poplars has considerably decreased. In the interest of forest stands stabilization in the lowland regions it will be necessary to:

- continue in the preservation of the most valuable individuals from a genotype point of view
- dedicate greater attention to and at the same time increase the area of gene bases
- increase the number of regionalized clones, minimally 2 clones within 3 years
- continue intraspecific hybridization with emphasis on stem plasticity, growth intensity and resistance to biotic and abiotic factors where appropriate
- proceed to the establishment of multiclonal stands within 5 years
- participate in clonal archives with clones from Central and Eastern Europe.



## The status of black poplar (*Populus nigra*) in Moldova

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The forests of Moldova occupy 9.3% of the country's territory. The natural zonal forests consist of *Querceta pertraea*, *Querceta pubescentis*, *Querceta roburis* and *Fageta sylvaticae* formations. The azonal forests are represented by *Saliceta albae* and *Populeta albae* typological formations.

The surface of the poplar forests in Moldova is 4920.9 ha. It makes up 2.4% of the whole surface of our forests. The natural poplar forests occupy 2559.5 ha or 60.1%. The forest plantations spread on a surface of 1961.4 ha.

Four species of poplar occur in the natural forests: *Populus alba*, *Populus canescens*, *Populus tremula* and *Populus nigra*. Most of this surface is covered with *P. alba* - 2999.8 ha (61.0%). *Populus nigra* occupies a surface of 486 ha or 10% of the total area covered with poplar forests. Up to now, 187 ha of *P. nigra* natural forest have been preserved and 299.6 ha of poplar stands have been planted. At the same time 1162.0 ha of *Populus deltoides* and 72.8 ha of *Populus pyramidalis* have been planted (Table 1). The poplar plantations show very good quality and growth properties: 11 m<sup>3</sup>/ha at the age of 30 years.

**Table 1. Surfaces occupied by different species of poplar in Moldova.**

Species	Natural forests (ha)	Planted forests (ha)	Total		
			ha	%	
<i>Populus alba</i> L.	2589.2	410.8	2999.8	61.0	
<i>Populus nigra</i> L.	187.0	299.0	486.0	10.0	
<i>Populus tremula</i> L.	183.3	17.0	200.3	4.0	
<i>Populus deltoides</i> March	–	1162.0	1162.0	23.6	
<i>Populus pyramidalis</i> Rosier	–	72.8	72.8	–	
Total:	ha	2959.5	1961.4	4920.9	100.0
	%	60.1	39.9	100.0	–

*Populus nigra* is found in the natural waterside forests of the Nistru and Prut river valleys (Fig. 1). Solitary examples have been found in moist locations at the sources of small rivers (tributaries of the Nistru and Prut): Bic, Botna, Isnovet and Cogilnic.

*Populus nigra* grows in moist places with a medium and short duration of flooding, on alluvial soils. It is not found in places with salty soils. It is a component of the willow (*Salix alba*, *Salix fragilis*) and *Populus alba* forests and more seldom is found in oak (*Quercus robur*) forests. In these types of forests *P. nigra* is characterized by an insignificant abundance (1-2, according to the Hult scale). It rarely dominates. The period of vegetation (with leaves on the trees) is from the second half of April to November. Natural regeneration in populations is very weak (Postolache 1995).

Up until 1958 plantations of *P. alba*, *P. nigra*, *P. deltoides* and *P. pyramidalis* had been created. After 1958 new species of poplar were introduced. Work on the selection and hybridization was begun, as well as the acquisition of new sorts of poplar at the Forest Station in Bender. This work, performed by G. Nicanorov (1970), indicated fast growth and resistance to pests and diseases under different environmental conditions. This study also aimed to obtain types of poplar with decorative qualities for use in urban plantings.

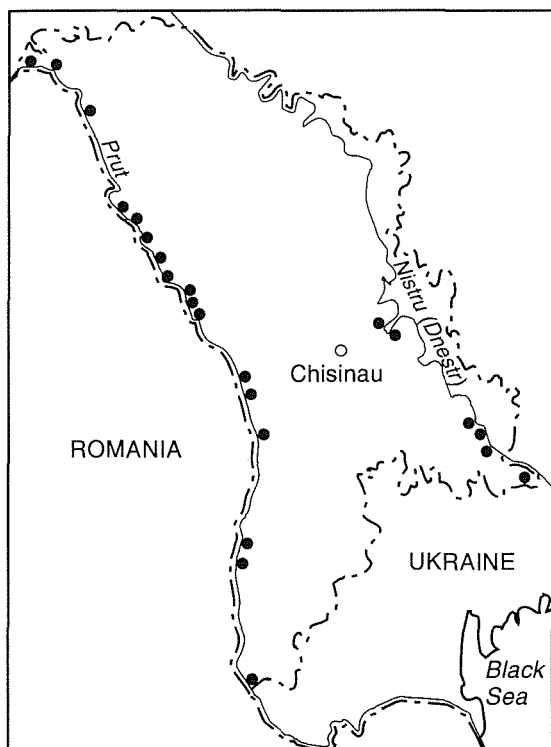


Fig. 1. The distribution of *Populus nigra* in Moldova.

From different places in Moldova, 165 parent trees (65 female and 100 male) were chosen: *P. alba*, *P. deltoides*, *P. bolle*, *P. pyramidalis* and *P. nigra*; 293 crossings were made. The hybrids obtained from these crossings showed good growth with the exception of *P. nigra* and *P. bolle*. The *P. nigra* hybrids are very different morphologically. It was observed that from the crossings of *P. nigra* with *P. deltoides* and *P. pyramidalis* the majority of the offspring had qualities most like those of *P. nigra*. In crossing *P. nigra* with *P. pyramidalis* hybrids, all the stages from a pyramidal crown to a brunchy one were obtained. All the hybrids of *P. nigra* evidenced an improvement of the trunk quality. As a result of this work, four types of poplar were obtained: Nistrean, Merenesti, Bender and Moldovenesc.

The elite samples of these kinds of poplars reach the height of 16-18 m at the age of 9 years and have a diameter of 15-16 cm. The annual growth increment is 1.8-2.1 m.

The actual state of many poplar plantations is unsatisfactory, as many of them were created in unfavourable conditions. As a result, many poplar plantations from the Prut river waterside are attacked by diseases at present.

The investigations of the genetic resources of *Populus nigra* are stopped because of certain difficulties, but at the same time it must be mentioned that this problem is considered serious in our country.

We have specialists who can undertake the following work:

- inventory of the forest resources
- the investigations of genetic diversity in different ecological conditions
- identification and preservation of the precious plantations and natural populations
- creation of new clones, sort collections, etc.

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## Black poplar in the Russian Federation

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### Range of black poplar in Russia

The range of distribution of *Populus nigra* in Russia occupies the European part, its central and southern regions. Black poplar reaches to the north along the Severnaya Dvina river to 63°N latitude (Archangelsk), but also grows in the Northern Caucasus, and in Siberia, at the shores and in the lowlands of the Ob, Irtish, Tobol, Abakan, Enisei rivers and their tributaries (Fig. 1). Black poplar reaches 1500 m asl in mountain regions. In lowland forests, poplar grows on alluvium, on silty, sandy soils and on drained peat soils. Black poplar inhabits emerging sandy and drift-gravel soils and promotes the deposition of alluvium and the formation of islands.

The lowland poplar forests are very dense, especially in juvenile stages, because herbaceous vegetation is nearly absent. With gradual thinning, species such as *Agrostis alba*, *Agropyron repens*, *Bromus inermis*, *Inula britannica*, *Lythrum salicaria* and *Mentha arvensis* appear. In black poplar stands *Salix alba* is often present and sometimes *Populus alba*. Poplar forests tolerate up to 2 months of spring flooding. The considerable stand density and close herbaceous cover prevent seed regeneration and the yearly alluvial deposits lead to hydrology changes which promote the invasion of *Ulmus laevis* and *P. alba*, and a gradual shift from black poplar stands to elm and white poplar forests. In less-flooded lowlands, black poplar stands develop with a rich layer of shrubs (*Prunus spinosa*, *Rosa cinnamomea*, *Rhamnus cathartica*, *Ribes nigrum* and *Tamarix*). The highest lowland sites are characterized by developing black poplar stands with *Calamagrostis epigeios* prevailing in the layer of herbaceous vegetation.

In the southern part of black poplar's range, on sandy dry soils, the stands form with *Tamarix laxa* and *Tamarix ramosissima*. Under human influence, natural black poplar stands with *Bromus* and *Calamagrostis* in the herbaceous layer are often replaced by coppice stands of black poplar with *Glycyrrhiza* and *Carex* spp. in the herbaceous layer. In the lowlands of the Ural river there are such coppice black poplar stands, with a well-developed shrub layer (*R. cinnamomea*, *R. cathartica*) and *Rubus caesius*, *Aristolochia clematidis* and *Glycyrrhiza glabra* prevailing in the herbaceous layer. The species composition of black poplar stands in the lowlands of the European Dnepr, Don, Volga and Ural rivers is very close to the composition of black poplar stands in the lowlands of the Siberian Ob, Irtish and Enisei rivers: the prevailing species are *A. repens*, *B. inermis*, *A. alba*, *I. britannica* and *Lysimachia vulgaris*.

Black poplar can form a robust root system. With flooding and burial in sandy deposits it can develop adventitious roots and form a multi-layered root system. It reproduces well from root shoots, and seedlings perform well only on fresh, bare sandy soils as a pioneer of lowland forests. Because under the forest canopy, generative regeneration is absent, and vegetative regeneration is difficult, shifts of black poplar stands in the European part of Russia occur (to white poplar and elm stands) and in Siberia (to birch forest types, sometimes with an admixture of *P. alba*, *P. tremula*, conifers, willows and other species) or to lowland meadows (see Anon. 1977).

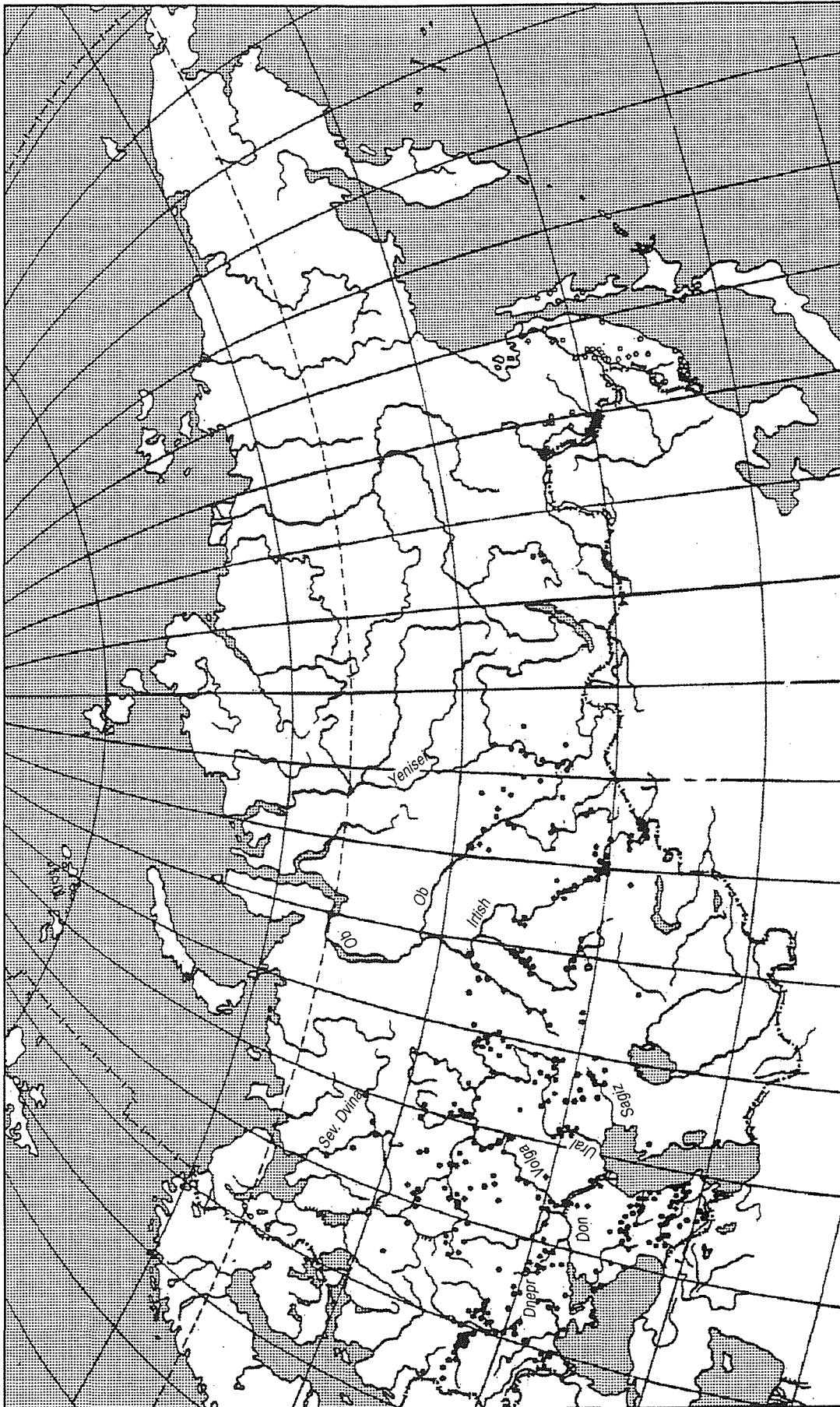


Fig. 1. Distribution of *Populus nigra* in Russia and the former USSR.

Black poplar is a fast-growing species, with a longevity of 200 years (according to some dates, even to more than 400 years; Shchepotiev and Pavlenko 1962). It is wind-resistant and tolerates higher flooding than *P. alba*. It reaches 30 m in height and a diameter of 1-2 m (up to 40-45 m high and 2-3 m in diameter) (Shchepotiev and Pavlenko 1962; Lavrinenko *et al.* 1966). This tree is anemo- and hydrochorous, more shade-tolerant than *P. tremula*, but less than *P. alba*; hydrophytic, oligotrophic, autochthonous species of lowland forests. It suffers less from heartwood rot than *Populus x euramericana*, but has relatively little salt resistance (Lavrinenko *et al.* 1966).

In the Caucasus, together with *P. nigra*, grows *P. nigra* var. *Sosnovskyi*, which was described by A. Grossheim as an independent species *Populus sosnovskyi*, but this was rejected by A. Macashvili as being within the range of the variety, the only different characteristic from black poplar being pointed tips of the leaves, which do not always occur. This variety grows at an altitude of 1200-1300 m asl in the river lowlands together with *P. alba*, *Ulmus carpiniifolia* and other species.

Black poplar is more resistant to different diseases and frost than other poplars. Stands occupy nearly 15 000 ha in river lowlands of Bashkiria. It is the fastest-growing species among native forest trees, and 40-year-old trees have been reported to have a productivity of 500 m<sup>3</sup>/ha. Individual trees at 33 years can reach more than 31 m in height; at 35 years, 36 m and at 70 years, 45 m.

In Bashkiria there is also a form of black poplar with yellow-grey bark, which grows considerably faster than the black-bark form, and at 40 years surpasses the latter by twice the volume and by a 31% greater diameter. In Tatarstan in the Vyatka river lowland, black poplar reached at 39 years of age the height of 34 m, diameter of 49 cm and the volume of 521 m<sup>3</sup> in a very dense stand. In the Volga river lowland (Ulyanovsk district) on the silt-sandy soils, at 32 years of age black poplar reaches 28 m height with 418 m<sup>3</sup>/ha of volume.

#### **Significance and role of black poplar in breeding in Russia**

Black poplar is widely used in different breeding programmes. Its wide compatibility with many other species threatens the existence of this species in some localities. Of worldwide catalogue of poplar clones, 63% derives from black poplar, mainly because of interspecific hybridization (Viart 1992).

Black poplar has been used for the breeding of poplars in the former USSR and in modern Russia. Many elite forms and varieties of poplars were created by the breeders A.B. Albensky, P.L. Bogdanov, A.M. Berezin, A.S. Yablokov, I.A. Kazartsev, S.P. Ivannikov, G.P. Ozolin, N.A. Konovalov, P.D. Besschetnov, N.W. Starova, A.P. Tsarev and others.

According to research by W. Bakulin (1990), black poplars reproduce with difficulty by vegetative sprouting, so for forest establishment, shores, canals and ravine reforestation seedlings are used. S. Ivannikov (1980) found and selected the forms of black poplar that successfully reproduce vegetatively. For planting in towns and settlements, the fast-growing forms are used, which are reproduced by green shoots. Black poplar is moderately resistant against SO<sub>2</sub>, NO-NO<sub>2</sub> and NH<sub>3</sub> (Nikolaevsky 1979).

Of the collections of poplar at introduction centres, black poplar is present in Altaj botanical garden, in Leninogorsk, Barnaul, Gorno-Altajsk, Omsk, Novosibirsk, Tomsk, Krasnojarsk, Abakan, Irkutsk and others. Intersection hybrids, such as *P. nigra* x *P. berolinensis* 5, *P. nigra* x *P. suaveolens* 85, *P. nigra* x *P. berolinensis* 165, *P. nigra* x *P. balsamifera*, are held in Altaj and Novosibirsk. Their biology is studied in a series of forest culture tests.

The breeding of poplar was started in Russia in the 1930s. It was observed that from the wood of the hybrid *P. nigra* × *P. berolinensis* 5 it is possible to produce matches of as high quality as from the wood of *P. tremula*; the hybrid *P. balsamifera* × *P. nigra* gives the best quality veneer. Hybrids of A. Berezin *P. nigra* × *P. suaveolens* 85 and *P. nigra* × *P. berolinensis* 5 are recommended for establishing forest cultures in the zone of broadleaved forests; the same hybrids and *P. nigra* × *P. pyramidalis* 180 are recommended for cultivation in the zone of forest-steppe, and *P. nigra* × *P. berolinensis* 5B in the steppe zone (Ivannikov 1971). As a result of breeding work by A.S. Yablokov on *P. pyramidalis* × *P. nigra*, Pioneer, Russky and Michurinets were obtained, which are used in towns and settlements in the European part of Russia. The hybrid *P. trichocarpa* × *P. nigra* was called one of the best poplars for the Moscow region (Kazartsev 1965).

In Siberia the mutants of black poplar are produced treating its seeds with X-rays. The numerous genetic deviations obtained are widely used for hybridization, with selection of prospective forms for hybrid testing. Black poplar has the somatic chromosome number  $2n=38$  by haploid number  $n=19$ . But there is also known a natural polyploid with the type  $3n=57$  (so-called triploid black poplar). Black poplar also is used for generating the artificial polyploidy.

In connection with the fast growth and resistance of black poplar against atmospheric pollutants, its use in the afforestation of industrial regions increases, as black poplar in those conditions maintains its properties better than other woody plants. So, in Tomsk district, only black poplar was recommended for afforestation of northern areas (Bakulin 1990). Black poplar is included in the afforestation for the Krasnojarsk, Altaj, Novosibirsk and Hakasia regions. With a sufficient quantity of available moisture in the soil, black poplar and its hybrids performed well in agricultural and protective afforestation (types Pioneer, Michurinets, *P. nigra* × *P. berolinensis*), surpassing *Populus balsamifera* by a growth increase of 6-20% (Bakulin 1990).

*Populus nigra* var. *italica* is one of the two most widespread pyramidal poplars in Russia. Although the name seems to indicate an Italian origin, its real origin is unknown. It probably derives from a spontaneous mutation of *P. nigra*, [which] occurred in central Asia. It was introduced in Italy in the 18th century, and from the Po Valley it was spread all over the world (from which the common name Lombardy Poplar (Cagelli and Lefevre 1995). In the Northern Caucasus it reaches a height of up to 35 m; it is a fast-growing, but a warmth-loving tree, not sufficiently frost-hardy. In the natural forest stands this poplar is absent. In contrast, the native black poplar can tolerate  $-40^{\circ}\text{C}$  and relatively inclement conditions and during a short summer can reach very good growth. For example, in Bashkiria near the city of Ufa, black poplar at 40 years reaches the height of 41 m. It reaches similar sizes on podzolic loamy soil near Moscow too, and has a high viability and longevity (Yablokov 1956).

### **Conservation role of black poplar**

Besides being of breeding significance, black poplar plays a nature-protective role in Russia. It protects the soils of the river lowlands, slopes and bottoms of ravines against erosion, is used in agricultural afforestation (in pure form and as a component of respective hybrid sorts and forms), has water-protective significance and participates in the formation of poplar stands in the Astrakhan semi-desert, which are irrigated with waste effluent from the Astrakhan pulp plant.

Black poplar, with its robust root system of many branch roots and offsets, is successfully used to create water-protective plantations in the river lowlands, shores of lakes and basins. In Bashkiria and on the Don, selected forms of black poplar, which are fast-growing, with a good stem form and high quality of wood, are used for mass-propagation and creation of productive plantations and for breeding. K.B. Losicky (1955) demonstrated that productivity of black poplar stands in the regions of Low and Middle Volga is not within the traditional, generally accepted quality scale of the site: its growth follows a line which surpasses the highest capacity class that sharply differentiates black poplar from all the other trees.

In Kamishin (Volgograd region) A.W. Albensky selected and successfully used in protection forestry the hybrid *P. nigra* var. *pyramidalis* × *P. nigra*. A.S. Yablokov, from the same crossing system, developed the types Russky, Michurinets and Pioneer, characterized by fast growth, cold-resistance and decorativeness. A.M. Berezin in Bashkiria created the hybrid *P. nigra* × *P. berolinensis*, which produces a volume 1600 m<sup>3</sup>/ha at 30 years. At 10 years of age, poplar variety Russky, made by A.S. Yablokov, in the forestry enterprise 'Obojan' in the central part of the forest-steppe zone had a mean height of 18 m, mean diameter of 18 cm and volume of 300 m<sup>3</sup>/ha. Black poplar is most used for protection in the regions of Middle and Low Volga and Don rivers, North Caucasus, Central Chernozem zone and in Bashkiria.

The research institute of forestry (VNIILM) collected more than 300 species, hybrids, types and clones of poplar; they were propagated and provided to 44 forestry enterprises and to forestry research stations of Bashkiria, Tatarstan, Don, Northern Caucasus and Astrakhan, for establishing mother plantations and for testing. Black poplar from Bashkiria was used by breeders A.S. Yablokov and A.M. Berezin to develop exclusively fast-growing hybrids. Thanks to poplar plantations, the microclimate near the city of Astrakhan is changing, the city is protected against the hot winds which often carry scorching sand, and a beautiful recreation zone has been established (Ivannikov 1980).

Black poplar drains the inundated lands due to enhanced transpiration through the canopy, prevents the irrigated fields from over-watering, strengthens the shores of rivers because of its high rooting offspring capacity and low demand for soil fertility; so it occupies one of the main positions in the afforestation of water-eroded waste lands and ravines in urban and production forestry.

#### **Current status and problems of conservation of the black poplar gene fund**

Black poplar occupies 250 000 ha of forest territory in Russia. The work for target gene resource preservation of this tree species in Russia now is absent, but the above-mentioned preservation objectives are being achieved by the presence of poplar stands in some localities. In the bottomlands of Volga and Ahtuba rivers in the Volga delta the native black poplar stands (originated from seeds) at 25-40 years have a volume of 230-300 m<sup>3</sup>/ha, with a mean annual increment of 7.5-16 m<sup>3</sup>/ha; the maximum occurs before 30-35 years (Ivannikov and Kazantsev 1975).

Black poplar tolerates well long flooding (up to 80 days), but in this case its productivity decreases by 20-40% compared with stands exposed to short- and medium-duration flooding. Black poplar is resistant against chlorine-sulphate salinization (Kazantsev 1973). In the region of Orenburg the general area of bottom land forests is 146 000 ha, mainly in lowlands of the Ural and Samara rivers. There, the poplar stands, mainly black and white ones, occupy 44 700 ha. Also occurring there are plantations of balsam poplar, introduced in the 1930s (Kazantsev 1988).

The natural distribution of black poplar on the Low Volga was restrained

formerly by the long flooding, but now, in connection with the regulation of Volga river, the duration of inundation is shortened by 1.2-3.0 times, and varies from 40 to 75 days. Black poplar tolerates such flooding well. Ecologically suitable for the growing of poplar are 54 500 ha of area in the Volgo-Ahtuba bottomland, but only 30 400 ha are occupied (Kazantsev 1970).

Preservation of genetic resources of black poplar is also attained through stands in nature reserves, forest preserves, forest genetic reserves and national parks. There are 31 national parks in Russia, 29 of them under the Forest service, with the total area of 6 536 100 ha. Some of these parks also contain stands of black poplar.

The national parks are:

- Kenozersky (region Archangelsk, 139 200 ha)
- Meshchersky (region Rjazan, 103 000 ha)
- Marij-Chodra (republic Marij-El, 36 800 ha)
- Bashkiria (republic Bashkortostan, 98 400 ha)
- Orlovskoje Polesje (region Orjol, 77 700 ha)
- Chavash-Varmane (republic Chuvashija, 25 300 ha)
- Zjuratkul and Taganaj (region Cheljabinsk, 86 700 and 56 400 ha, respectively)
- Nizhnjaja Kama (republic Tatarstan, 26 100 ha)
- Valdajsky (region Novgorod, 158 500 ha)
- Meshcheri (region Vladimir, 118 800 ha)
- Smolny (republic Mordovija, 36 500 ha)
- Samarskaja Luka (region Samara, 134 000 ha)
- Hvalinsky (region Saratov, 25 500 ha)
- Sochinsky (region Krasnodar, 190 000 ha)
- Prielbrusje (republic Kabardino-Balkarija, 100 400 ha)
- Shorsky (region Kemerovo, 418 200 ha)
- Shushensky Bor (region Krasnojarsk, 39 200 ha).

The nature reserve Astrakhansky has 80 200 ha, including 32 000 ha of forested area, 13% of which contains black poplar, with the mean volume of 116 m<sup>3</sup>/ha.

Black poplar is preserved also in the forest compartments and on the plots, which are used for breeding programmes, as seed stands and plus trees. There are 54 plus trees and 19 ha of sustained seed plots of black poplar in the Povolgsky economical district, Astrakhansky region, in the Astrakhansky seed-breeding centre; 9 plus trees were certified in 1995. All trees were selected by phenotype. They grow in forest enterprises Ahtubinsky, Enotaevsky, Harabalinsky, Narimanovsky and Chernojarsky, and are present in artificial stands, coppice forests and seedling stands. Their age varies from 22 to 55 years, height is 18-36 m, diameter is 20-90 cm, the bark colour is from light to dark grey, the bark has light to middle 'cracking'. There are similar breeding stands in some forestry enterprises in Kursky, Belgorodsky, Orlovsky, Orenbourgsky and other regions.

There are some unique native stands of black poplar in Russia, characterized by high productivity and stability and having unique potential for breeding and cultivation. Preventing the loss of this valuable genepool is of great interest not only for Russia, but for all mankind too.

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## Diversity and population biology of *Populus nigra* L.: relevant issues to the conservation of genetic resources

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This contribution summarizes the main results of a doctoral thesis (Legionnet 1996), which was aimed at providing guidance to the conservation of genetic resources of *Populus nigra* in France. The results allowed us to define guidelines for strategies of sampling of genetic resources, and we also derived some precision useful for *in situ* conservation. In the first part of this presentation, I will report our results about genetic diversity and its organization, and discuss their consequences on sampling strategies. In the second part, I will discuss the possible evolution of genetic diversity.

### Present genetic diversity: consequences for sampling strategies

Two different aspects of diversity were studied: first, the amount and organization of genetic diversity at isoenzyme loci, and second, the organization of diversity of an adaptive trait: the susceptibility to the foliar rust fungus *Melampsora larici-populina*. This study was carried out on a collection of clones mainly representing France (Fig. 1).

### Neutral diversity: results obtained with isozymes

The experiments and results are described in part in Legionnet and Lefèvre (1996). The isozymes were extracted from buds and young leaves, and separated and stained on starch gels. Eight Mendelian polymorphic loci were scored. The amount of genetic diversity was assessed using the gene diversity index  $H$  as described by Nei (1973). An unbiased estimate of  $H$  and the variance of this parameter were computed according to Weir (1990). The results obtained over the *P. nigra* collection are shown in Figure 2, and comparisons with other species are listed in Table 1. In the *P. nigra* collection, the highest diversity is held by the set of clones representing foreign countries. Although little is known about the origin of these clones, this result suggests that gene diversity may be impoverished in the marginal part of the species range represented in France. Contrasted values are obtained for the different regions and stands, and great differences appear between neighbouring stands. Compared with the other angiosperm trees, *P. nigra* displays a gene diversity slightly above the mean.

The differentiation was measured with Nei's  $G_{ST}$  index (1973), computed according to Nei and Chesser (1983) (Table 1). This index measures the proportion of diversity contained among divisions. Like the other species of the same family, *P. nigra* displays a low differentiation compared with the other angiosperm trees. Furthermore, angiosperm trees display a low differentiation compared with other plant or animal species.

The high level of gene diversity and the low differentiation found in tree species is usually explained by considerable geneflow (Kremer 1994). In *P. nigra*, seeds and pollen are very small and easily dispersed, so that this explanation could be acceptable. However, we observed some features that make it questionable. Indeed, some alleles were found fixed in some places. The best example is the allele *SDH-3*, which is the only allele found at this locus in the stand Mallemort (over 50 trees and 11 maternal progenies representing 200 individuals), whereas this allele is present in a site of the same river valley 10 km apart. These observations suggest indirectly that geneflow may be limited in this species.

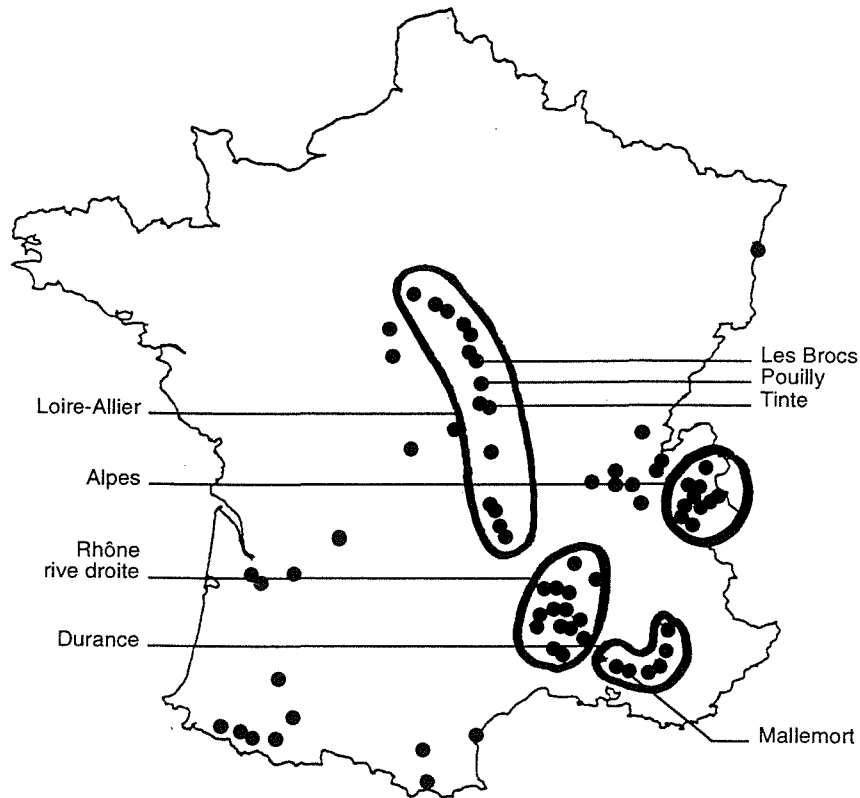


Fig. 1. Collection of *Populus nigra* clones used for the evaluation of gene diversity at isoenzyme loci.

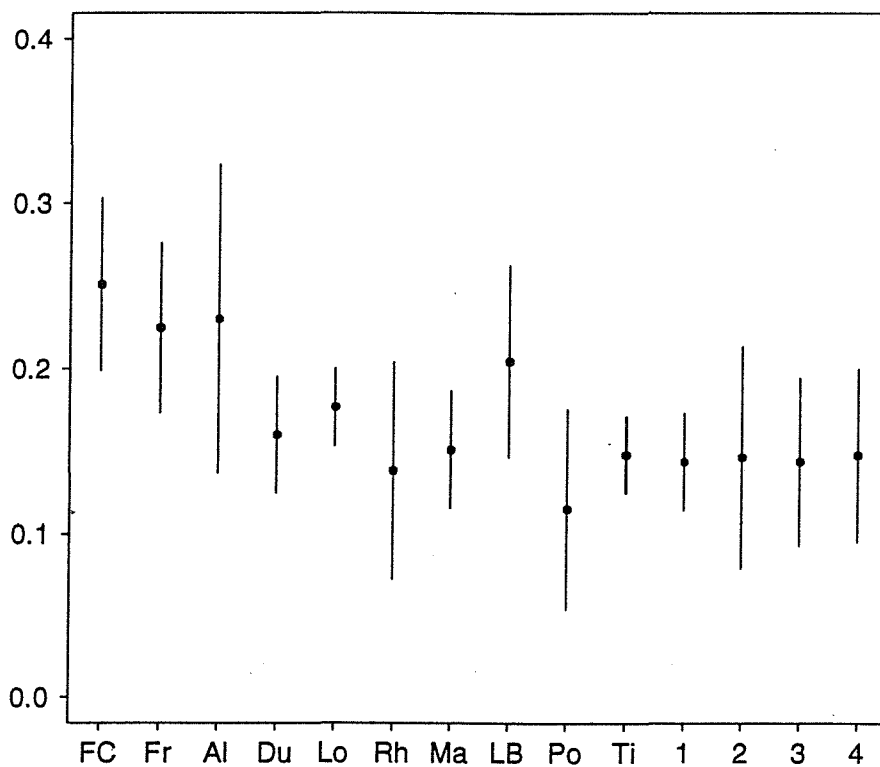


Fig. 2. Gene diversity index with 95% confidence interval over the *Populus nigra* collection. FC=Foreign Countries; Fr=France; Al=Alpes; Du=Durance; Lo=Loire; Rh=Rhône; LB=Les Brocs; M=Mallemort; Po=Pouilly; Ti=Tinte; 1 to 4 = zones 1 to 4 in Tinte.

Table 1. Gene diversity index (H) and differentiation index ( $G_{ST}$ ) for *Populus nigra*, and comparison with other species.

	H (Nei 1973)	$G_{ST}$ (Nei and Chesser 1983)
<i>Populus nigra</i>	Stands: 0.115 to 0.204 France: 0.198 Other countries: 0.251	Between stands: 0.063 Between regions: 0.033
Mean of angiosperm trees	0.15	0.11
<i>P. tremuloides</i>	0.22 to 0.29	0.068
<i>P. trichocarpa</i>	0.07 to 0.11	0.063
<i>Salix viminalis</i>	0.12 to 0.24	0.072
<i>Alnus glutinosa</i>	0.28	0.17

Another point of interest is that the highest diversity found among stands was in Les Brocs, which is the stand where the oldest trees grow. This may be explained by natural selection favouring the most heterozygous genotypes. The hypothesis that the age of the trees found in one stand may influence the level of gene diversity should be further investigated.

#### Organization of variation of adaptive traits

A subset of the previous collection was used to study adaptive traits related to susceptibility to the foliar rust *M. larici-populina* (Legionnet *et al.* unpublished)<sup>1</sup>. The field susceptibility was measured in one field trial, and susceptibility was split into simple epidemiological components in laboratory tests. Monoracial inocula of races E1, E2 and E3 of the pathogen were used, as well as controlled growing conditions. Seven components of susceptibility were measured on each individual: size of uredia for the three races, number of uredia after one cycle of the fungus for the three races, and length of the infectious cycle for the race E1. The main results of this study are:

1. The independence of the components of susceptibility, revealed by multivariate analysis. Indeed, similar levels of field susceptibility were found among individuals displaying very contrasted combinations of the individual components of resistance.
2. The very low correlation between the components of rust susceptibility and geographical or meteorological data.
3. The existence of significant differences between regions and between stands, revealed by the analysis of variance; but the proportion of variation found at the different hierarchical levels is very variable (Table 2).

These results show that adaptive traits may show higher levels of differentiation among populations than neutral traits, which confirms the relative restriction of geneflow. The differences in the scales of differentiation can be explained by differences in the time of appearance and rate of expansion of new, favourable gene or gene combinations.

#### Consequences for sampling strategies

In both analysis of neutral and adaptive diversity, we showed that most of the genetic variation was within stand; a considerable part of variation is at the level of individuals due to the high heterozygosity (which is close to gene diversity for outcrossing species). As a consequence, in the sampling of genetic diversity it

<sup>1</sup> Legionnet, A., H. Muranty and F. Lefèvre. Unpublished. Genetic variation of the riparian pioneer tree species *Populus nigra* L. II. Variation in susceptibility to the foliar rust *Melampsora larici-populina* Kleb.

**Table 2. Organization of the genetic variance of adaptive traits at two hierarchical levels.**

	Part of variance between region (%) $\sigma^2 \text{ region}/(\sigma^2 \text{ region} + \sigma^2 \text{ clone (region)})$	Part of variance between stands (%) $\sigma^2 \text{ stand}/(\sigma^2 \text{ stand} + \sigma^2 \text{ clone (stand)})$
Field <sup>†</sup>	21	10
1	16	22
2	3	0.4
3	10	13
4	17	0.6
5	2	8
6	18	18
7	20	17

<sup>†</sup> Field=field susceptibility; 1 to 7=seven epidemiological components of susceptibility measured in laboratory tests.

would be more efficient to collect a large number of individuals on a small number of stands rather than to prospect a large number of stands.

The indication that heterozygosity may increase in ageing stands is in agreement with results reported for other species, and with a study of the evolution of gene diversity at young stages (Legionnet 1996; Legionnet and Lefèvre, unpublished<sup>2</sup>). As a consequence of this phenomenon, more diversity could be collected by sampling the oldest trees.

The restriction of geneflow revealed in our study and the organization of adaptive variation show that some differentiation may exist, but the scale of differentiation is difficult to predict and may vary from one trait to another. Collecting germplasm should also take into account the potential local adaptations; thus an efficient sampling strategy should also consider the various geographic origins. The relative effort dedicated to within- and among-stand sampling still needs to be clarified.

#### **Possible evolution of gene diversity**

The evolution of genetic variation in a species depends on many intrinsic and external factors. The intrinsic factors are related to the biology of the species, e.g. mating system, migration, vegetative propagation, etc. The external factors are the interaction of the species with its environment, and in particular with other species (pathogens, competitors), and with humans (fragmentation of the species range, ageing of stands, decreasing regeneration, etc.). These are mainly factors affecting the demography. To predict the evolution of gene diversity, we need to use a model in which it will be possible to include as many factors as possible. Among the models available in population genetics, the only one that allows us to take into account genetic and demographic factors is the Metapopulation model.

#### ***The Metapopulation model***

In this model, a species is distributed on a fragmented range on N sites, each bearing a subpopulation. At any time, a site can bear a subpopulation, or be empty and available for colonization. Any population has a restricted duration, between its foundation and its extinction. Between these two events, the subpopulation undergoes a demographic evolution, which implies that the subpopulations are not a demographic equilibrium. This is the major originality of this model, and this is

<sup>2</sup> Legionnet, A. and F. Lefèvre. Unpublished. Genetic variation of the riparian pioneer tree species *Populus nigra* L. III. Evolution at early stages of natural stand installation.

what allows us to take into account demographic parameters, and to study genetic traits for which natural selection varies at different demographic stages. Migration events between subpopulations may be included in this model.

Because it is very complete compared with other population genetics models, the Metapopulation model seems to be what will be needed to predict the evolution of gene diversity in a species. However, the use of this model requires a knowledge about characteristics of the species biology that may be difficult to have. As pointed out by Slatkin (1987), knowledge about colonization and extinction events may be very difficult to establish, because these events are rare and mostly due to chance. To use the Metapopulation model for *P. nigra*, here are some questions that we should be able to answer: (i) What is the size of a subpopulation? (ii) What is the amount of migration? (iii) How does the demography evolve? (iv) How do foundation and extinction events occur?

Some preliminary results were obtained in the work of Legionnet (1996).

#### ***Observations and experimental results about the species biology***

**Foundation events and evolution of gene diversity.** Observations were done in a natural stand in Beaugency (Loire River valley) (see Legionnet and Lefèvre, unpublished<sup>2</sup>). In this stand, cohorts of various age classes could be found, and their genotypic composition was compared using four isozyme loci. The main results are as follows.

We showed that the genotypic composition of seedling patches could be explained only by the participation of several male, and, in most cases, several female parents. Moreover, the establishment and survival of seedlings requires particular conditions of soil and water availability. The fluctuation of these conditions at a microgeographic scale (favourable sites may be as small as several square centimetres), together with the considerable differences in time of seed maturity between female trees, ensure that the recruitment of seedlings in a site is constituted of various origins.

Although vegetative propagation is frequently observed in this species, we showed that adult stands were mostly of sexual origin and were mostly composed of different genotypes. This result was obtained in an adult cohort in Beaugency using DNA markers, and confirmed with isozyme markers on four other stands.

This result was also obtained in Beaugency with the comparison of four age classes; it needs to be confirmed, because only two isozyme loci could be used for this purpose. However, we showed a significant increase of gene diversity between 1- and 2-year-old seedlings.

**Gene flow.** As a consequence of these experimental results, some knowledge may be derived about gene flow: various origins contribute to the foundation of one stand (not only the nearest neighbours provide seeds that settle in a given site), and crossings are not limited to the nearest neighbours (this is due to considerable differences in flowering phenology). The distance of dispersal of seeds and pollen remains an important question that should be answered.

#### ***Evolutionary perspectives***

We made an evaluation of the present gene diversity that will be a reference point to study the further evolution of diversity in France. A similar study was done in Italy (Malvolti, pers. comm.), and gene diversity appears to be comparable in these two countries. A comparison with the other parts of the species range may be useful to detect possible zones of higher or lower diversity.

The further evolution of diversity could be predicted using a complete population genetics model, e.g. the Metapopulation model. For this purpose, more knowledge is needed about the different factors that may affect this evolution, in particular intrinsic factors like the scale of geneflow and the inbreeding depression, and external factors like the foundation events and the fragmentation of populations. This last factor should be of considerable importance, since *P. nigra* is a highly heterozygous species, and is therefore very likely to bear a heavy genetic load (because detrimental alleles are not eliminated by natural selection when they are 'masked' by another allele in heterozygotes). If the population size decreases, because of the fragmentation of the area and the restriction of geneflow among local populations, the inbreeding will increase. If the species has a strong inbreeding depression (as we can suppose owing to the genetic load), the fitness would then be affected and the species may be unable to support competition. Therefore the main questions that should be answered about the biology of the species are the scale of gene exchange (what density of stands would allow the local populations to exchange genes?) and the intensity of inbreeding depression.

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This review aims to be almost exhaustive on *Populus nigra*, with regard to the conservation of genetic resources. Some chapters also deal with related poplar species, as mentioned in the titles, but most of the literature concerning hybrid poplar breeding was discarded. Simple summaries were not retained. More general references about genetic resources should be found elsewhere.

Language is indicated for each reference (underlined> and summaries. Titles were eventually translated in English, French or Spanish and given in [ ].

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## Programme of the meeting

### Saturday, 5 October 1996

- 0800 Departure from Budapest by bus (Bus session: Review of literature)  
 1100 Arrival in Sárvár, accommodation  
 1200-1300 *Lunch*  
 1330-1400 Introduction  
 1400-1600 Brief presentations of the progress made in the national programmes since the last meeting  
 1600-1630 *Coffee break*  
 1630-1900 Genetics and population biology of *Populus nigra*; consequences for practical gene conservation strategies  
 1900-2000 **Update of the workplan (session I). Tasks:**  
*Ex situ* gene conservation methods in *Populus nigra*: common guidelines (S. Bisoffi/L. Cagelli)  
 2030 *Dinner*

### Sunday, 6 October 1996

- 0800-1000 **Update of the workplan (session II). Tasks:**  
 Exchange of reference clones (J. Van Slycken)  
 EUFORGEN clone collection update (S. Bisoffi/L. Cagelli)  
 Database of the European collections (S. Bisoffi/L. Cagelli)  
 1000-1030 *Coffee break*  
 1030-1300 **Update of the workplan (session III):**  
 Synthesis of current *in situ* conservation measures and activities (S. de Vries)  
 Standardized list of descriptors for *Populus nigra* stands /stand typology  
 Discussion and agreement on other Network tasks and activities  
 1300-1400 *Lunch*  
 1400-1700 Excursion: Experimental plantations maintained by the Sárvár Forest Research Station  
 1700-1730 Building up of a national programme on forest genetic resources: the experience from Hungary  
 1730-1800 Presentation of experimental results from the Sárvár Research Station  
 1800-1900 Presentation of the joint "EUROPOP" research project proposal, discussion of further research needs and plans  
 1900-2000 Broadening of the scope of the Network: discussion  
 2000-2100 *Dinner*  
 2100-2200 Communication, mailing lists  
 Election of the Chair  
 Next Network meeting

### Monday, 7 October 1996

- 0730-1130 Field trip to *Populus nigra* stands (Bus session: Information from other EUFORGEN Networks)  
 1130-1300 Farewell *Dinner* at a hunting lodge  
 1700 Arrival in Budapest

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