The conservation of *Populus nigra* L. and gene flow within cultivated poplars in Europe

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Abstract

Cagelli, L. & Lefèvre, F.: The conservation of *Populus nigra* L. and gene flow within cultivated poplars in Europe. — Bocconea 7: 63-75. 1997. — ISSN 1120-4060.

Wild stands of *Populus nigra* L. can be considered on the verge of extinction in a large part of western Europe. Its natural habitat is being gradually reduced by human activity and the gene pool is threatened by the large-scale presence of cultivated hybrids and of one ornamental cultivar, the fastigiate 'Lombardy poplar', that spontaneously hybridize with *P. nigra*.
Although cultivars of *P. nigra* are cultivated in some areas, especially in the Middle East, the main use of this species is as a parent of interspecific hybrids in breeding programmes. From an economic point of view the most important hybrid combination is produced by using *P. nigra* as a pollen parent and with the North American *P. deltoides* Bartr. as the seed parent, resulting in the common 'euramerican' hybrids (*P. euramericana* (Dode) Guinier). Some varieties of black poplar are also widely used for their ornamental qualities. This represents an original example of a wild/cultivated complex pool in forestry.
The conservation of *in situ* genetic resources is limited to some restricted areas, such as the riparian forests, typical habitat of *P. nigra*, which have suffered considerably from urbanization, industrialization and competition with human productive activities. *Ex situ* conservation activities, on the other hand, have been carried out in several European countries. A project for the coordination of efforts aimed at the conservation of *P. nigra* has been recently launched as one of four pilot projects included in the European Forest Genetic Resources Programme (EUFORGEN).

Taxonomy of the genus *Populus* L.

The genus *Populus* L. belongs to the family *Salicaceae*; it includes (according to different taxonomists) between thirty and forty species, distributed in the temperate and cold regions of the northern hemisphere from 20° to 70° N latitude. *Populus* are typical pioneer species, clearly heliophilous, mostly dioecious and wind-pollinated.

The genus is generally divided into 5 sections (Houtzagers 1950, FAO 1980): *Turanga*, *Leucoides*, *Leuce*, *Aigeiros* and *Tacamahaca*; Eckenwalder (1977, 1995) proposed the

addition of the monotypic section *Abaso* while Kosla & Khurana (1982) suggested placing the Himalayan species *P. ciliata* Wall. (previously attributed to sections *Tacamahaca* or *Leucoides*) in a new section *Ciliata*.

The taxonomy of *Populus* is further complicated by a high proneness of its members hybridise with one another. Such interspecific hybridization is a natural process where the natural ranges of two species overlap (Barnes & Pregitzer 1985, Dickmann & Stuart 1983, Eckenwalder, 1984a, b, Keim & al. 1989, Muhle-Larsen 1970, Ronald & al. 1973a, b, Rood & al. 1986, Whitham & al. 1995). It is also brought about through controlled crosses (Ronald 1982, Stettler & al. 1980, Willing & Pryor 1976) or both, when species are introduced in cultivation outside their natural range (Spies & Barnes 1981, 1982, Eckenwalder 1982, FAO 1958, Ronald & Steel 1974). Hybrids exist both within and between the taxonomic sections (Rajora & Zsuffa 1984), and the production of some of them is favoured by pre-treatment of pollen or stigmas (Stettler & al. 1980, Gaget & al. 1989); three- and four-way hybrids including intersectional combinations have also been obtained by using interspecific hybrids as parents (Lemoine 1988, Rajora & Zsuffa 1984, Ronald 1982, Zsuffa 1974).

The flower biology of *P. nigra* has been extensively studied (Villar & al. 1987a, Label & al. 1994), and also its cross-incompatibility barrier with species from other botanical sections (Gaget & al 1989, Villar & al., 1987b, Villar & al. 1993). However, in the case of intrasectional hybridization with *P. deltoides*, although many attemps have been made to overcome incompatibility barriers in poplars such as pre-treatment of pollen or stigma (Knox & al. 1972, Whitecross & Willing 1975, Guries & Stettler 1976, Willing & Pryior 1976, Stettler & al. 1980), *in vitro* embryo culture (Li & al. 1983, Kouider & al. 1984, Li & Li 1985, Noh & al. 1986, Savka & al. 1987) and more recently also *in vitro* fertilization (Douglas, pers. comm.), it is still not possible to overcome the interspecific incompatibility in the cross involving *P. nigra* as female parent and *P. deltoides* as the male.

The characterization of species and interspecific hybrids and studies of introgression werelargely based in the past on morphological features (Ronald & al. 1973a, Eckenwalder 1982, 1984a, b). These recently received new attention when computers were used both for data acquisition and for the analysis of huge data sets (Bisoffi & Cagelli 1992, Hu & al. 1985, Rood & al. 1986). Biochemical markers are also in use: paperchromatography (Bortitz 1962, Boccone 1975, Malvolti & al. 1991) and gas chromatography (Baiocchi & al. 1990, Greenway & al. 1991, Ronald & al. 1973b, Ronald & Steel 1974) permit the discrimination of species and hybrids. Isozyme analysis can detect differences even between a limited set of clones (Malvolti & al. 1991, Rajora 1989a, b, c, Rajora & Dancik 1992).

Molecular techniques are now available and several have been used with poplars: ribosomal DNA (D'Ovidio & al. 1990, 1991, Faivre-Rampant & al. 1992a, b), mitochondrial DNA (Barrett & al. 1992), chloroplast DNA (Smith & Sytsma 1990), RFLP of genomic DNA (Keim & al. 1989), RAPD (Castiglione & al. 1993) and STS (Bradshaw & al. 1994, Faivre-Rampant & al. 1995). Ribosomal DNA polymorphisms have been suggested as suitable tools for detecting introgression of foreign germplasm into *P. nigra* (Faivre-Rampant & al. 1992a).

Although according to botanical classification *P. nigra* is included in Section *Aigeiros*, some recent systematic studies revealed ambiguity about the placement of the black poplar (Smith & Sytsma 1990, Faivre-Rampant & al. 1995).

The natural range of *P. nigra* extends over Europe (mainly central and southern), western Asia and North Africa. A wide range and a clear human intervention in the spread of the species makes the taxonomy of the species particularly complex: there are often several synonyms for the same variety and intermediate forms from spontaneous hybridization between varieties that make it difficult to classify in an unequivocal way. Zsuffa (1974) recognizes in a review the following varieties: *P. nigra* var. *nigra*, *P. nigra* var. *italica* Duroi, *P. nigra* var. *betulifolia* (Pursh) Torr., *P. nigra* var. *caudina* Ten. (= *P. nigra* var. *pubescens* Parl.), *P. nigra* var. *thevestina* Dode, *P. nigra* var. *neapolitana* Ten. (considered by Allegri 1956 and Gellini 1975 a hybrid *P. euramericana* (Dode) Guinier) and *P. nigra* var. *sinensis*. Bugala (1967) also considered *P. usbekistanica* Kom. and *P. sosnowskyi* A. Grossh as closely related to this species.

Ecology of wild *P. nigra*

P. nigra is a typical pioneer species growing in riparian mixed forests together with *P. alba* L., willows, alders, maple, elm, ash and, in more evolved forests, with oaks. In the colonization phases it follows the hygrophilous pioneer forests characterized by *Salix*. Although hygrophilous itself, *P. nigra* does not tolerate prolonged flooding. Heliophily and plasticity allow black poplars to settle also on poor soils and to colonize open areas on river banks. The dynamics of the populations and the different phases of colonization are directly related to the dynamics of the rivers and have been extensively described by Herpka (1986). From a genetic point of view Legionnet (1996) showed that colonization may occurr through patches of half sib seedlings.

Sexual maturity is generally attained at 6 to 10 years of age, but may be delayed by unfavourable environmental conditions. Flower buds differentiate at the end of the summer and flush before the sprouting of vegetative buds in late winter and early spring. The fruits ripen in late spring and early summer.

Both pollen and seed are produced in abundance, and both rely on wind for diffusion. Seed viability, initially high, decreases rapidly in 3-4 weeks in the open, although seeds can be stored at low temperature for some years (Muller & Tessier du Cros 1982).

Evidence of spontaneous vegetative propagation is commonly found at the juvenile stage in this species: fallen trees, broken roots and branches transported by the rivers can root very easily when partly planted in the soil; root suckers are also found. In a recent study Legionnet (1996) observed that although vegetative propagation was common in some young natural populations, in adult ones almost all the individuals sampled had different genotypes. Most of the genetic variation is found within stands or within rivers However some differentiation among populations may be detected and should be taken into account when collecting material (Legionnet & Lefevre 1996).

A study on the behaviour of *P. nigra* coming from various sources towards *Melampsora allii-populina* Kleb. and *Melampsora larici-populina* Kleb. showed a high variability among individuals within provenances, but limited differences among provenances (Cellerino & al. 1986). Legionnet (1996), however, found some small differences among stands were instead observed in France in the components of resistance to *Melampsora larici-populina*.

At present the wild black poplars can be considered on the verge of extinction in a large part of Western Europe. The reasons for their disappearance and reduction of their habitat

is human activity and widespread cultivation of the euramerican hybrids (*P. euramericana* (Dode) Guinier). Although gene exchange between species is a major event for evolution, the problem, in this case, is the fact that the genetic 'pollutants' (pure exotic species, their hybrids or *P. nigra* var. *italica*) represent a very narrow genetic base spread on a very wide scale (Arbez & Lefevre 1997, this volume).

Cultivation of pure *P. nigra*

P. nigra, like forest trees in general, has not been subjected to high degree of domestication as was the case of many agricultural crops. Thanks to its easy vegetative propagation and fast growth,the cultivation of this species was once common in some European countries. In Turkey *P. nigra* is still very widely cultivated: according to the latest inventory data it covers an area of 60 000 ha (as compared with 70 000 ha of euramerican clones) including both plot plantations and row plantations along water canals and stream banks. Black poplar clones "Anadolu" and "Gazi" are mainly cultivated in artificial plantations in Central and Eastern Anatolia. Some trials are now under way to select new clones with better growth performance and better resistance to frost damages. The wood is utilized as round wood for rural constructions and, like the euramerican wood, by the industries of plywood, furniture, packaging, particle boards and matches. In Spain the clonal cultivars "Bordils", "Poncella" and "Blanquillo de Granada" reached great popularity and large distribution at the end of the last century. Many more clones are included in the 'International Catalogue of Poplar Clones', prepared by Viart (1992) for the International Poplar Commission; however, there is scanty information about the actual scale of cultivation. No block plantations of pure *P. nigra* are reported at present in Croatia, France, Italy and Spain, although some *P. nigra* clones are included in the National Catalogues of Forest Clones and registered for commercial use in several European countries. *P. nigra* has been generally replaced by the more productive euramerican hybrids. Row plantations of *P. nigra* are common for wind-breaks in the Rhône valley in France. In the Netherlands *P. nigra* is still largely employed in roadside-plantation and has a limited use in plot plantations (Schalk 1983).

In the last years, however, a special programme was been developed to reintroduce this species along rivers. In Hungary *P. nigra* is used mainly in protected areas and flood-plains and covers an area of about 4 500 ha (3% of the total poplar area).

Ornamental varieties deserves particular consideration: *P. nigra* var. *italica* is largely used for wind-breaks and along roads all over the temperate regions of the world, while *P. nigra* var. *thevestina* (in particular the cv. "Hamoui") is widely cultivated in the Near East.

Use in breeding programmes

Like most poplar species, the European black poplar is cross-compatible with a variety of other *Populus* species. In fact many hybrid varieties involving *P. nigra* have been obtained. It is interesting to observe that 63% of the clones of the 'world' catalogue (International Catalogue of Poplar Clones - Viart 1992) descend from this species, mainly through interspecific hybridization (Table 1).

P. nigra has many desirable characteristics that determined its inclusion as parent in several improvement programmes going on in Europe: wide adaptability to many

environments and different kinds of soil, excellent rooting ability of stem cuttings, fair resistance to *Marssonina brunnea* (Ell. & Ev.) P. Magn., high level of resistance to bacterial canker (*Xanthomonas populi* Ridé) and mistletoe (*Viscum album* L.), and resistance (though probably not immunity) to PMV (Avanzo & al. 1985, Pichot & Teissier du Cros 1988, Sallé & al. 1991, V. Steenackers pers. comm.).

Table 1. *P. nigra* clones and hybrids included in the International Catalogue of Poplar Cultivars

P. nigra	"Anadolu"; "Ankum"; "Blanc de Garonne"; "Blanquillo de Granada"; "Bordils"; "Brandaris"; "Chile"; "Farsi"; "Fucini"; "Gazy"; "Hamoui"; "Irresheim"; "Italica"; "Jean pourtet"; "Loenen"; "Poncella"; "Sarrazin de Seilh"; "Schoorldam"; "Vereecken"; "Vert de Garonne"; "Wolterson"
P. deltoides × *P. nigra*	98 clones
P. nigra × *P. trichocarpa*	"Andover"; "Roxbury"
P. nigra × *P. laurifolia*	"Frye"; "Rumford"; "Strathglass"
P. maximowiczii (*P. laurifolia* × *P. nigra* var. *italica*)	"Geneva"; "Oxford"
P. tacamahaca (*P. laurifolia* × *P. nigra* var. *italica*)	"Maine"
P. maximowiczii × *P. nigra*	"Rochester"

P. × *euramericana* is the most common hybrid of *P. nigra*. It resulted from spontaneous hybridization occurred in Europe in the 18th century, between the American *P. deltoides* and the European *P. nigra*. These hybrids combine some favourable characteristics of the American species (fast growth, good wood quality, resistance to relevant leaf diseases) with the above-mentioned favourable traits of the European species. Their success in commercial culture was tremendous, especially in Southern Europe. For these reasons *P. nigra* is included in the breeding programmes of several European countries. The Italian programme, for example, which aims at improving *P. euramericana*, is been based on a semi-reciprocal recurrent selection of the parent species (Avanzo & al. 1985, Bisoffi 1989), although it is hampered by the incompatibility of the reciprocal cross *P. nigra* (female) *P. deltoides* (male) (Melchior & Seitz 1968).

Crosses with another American species, *P. trichocarpa*, have been made in Belgium and recently also in France. Although characterized by fast growth and resistance to *Xanthomonas populi*, the hybrids, obtained using *P. nigra* both as female and as male parent, are susceptible to other diseases (*Melampsora larici-populina* Kleb. and *Dothichiza populea* Sacc. & Br.) and display a strong tendency to produce epicormic shoots after pruning. Therefore, they have never been employed in commercial cultivation (V. Steenackers pers. comm.).

P. nigra has also been used for crosses with some Asiatic species of the section *Tacamahaca*. Well known is the clone "Rochester" obtained by the Oxford Paper Company (USA) from a cross between *P. maximowiczii* (female) and the European black poplar. Other hybrids of this kind were obtained both in Europe and in the United States (Table 1).

Hybrids between the Chinese *P. simonii* (female) and *P. nigra* are largely cultivated in China; however, the behaviour of these Euro-Asiatic hybrids in Europe is little known. Exploratory breeding with selected parents of *P. nigra* and *P. deltoides* is under way at the Istituto di Sperimentazione per la Pioppicoltura (ISP) of Casale Monferrato, Italy (Frison & Bisoffi 1988).

Some other simple or complex intersectional hybrids with the European black poplar are reported in literature: (Zsuffa 1974, Ronald 1982, Richens 1945 in Rajora & Zsuffa 1984, Starova 1977 in Rajora & Zsuffa 1984, Beatson 1991, Heimburger 1970, Zhao & Zhang unpublished data, Herpka 1960).

The conservation of genetic resources in Europe

The goals of the conservation of genetic resources are both to maintain a large gene pool for evolution that may ensure the potential for natural adaptation and to provide base material for further breeding operations.

In order to preserve the adaptability of a species the most advisable action to take would be the protection of its natural habitat (*in situ* conservation), so as to maintain the gene complexes that have evolved and that will evolve during time in response to enviromental changes. This kind of conservation, however, might not be applicable to *P. nigra* in large parts of its European range. Natural stands are often already considerably disturbed and fragmented. In addition they cannot be preserved from considerable gene flow from cultivars by the establishment of large protection bands.

Moreover, a riparian forest is a very dynamic ecosystem subject to rapid sequences of evolution stages and heliophilous pioneer species such as poplars tend to be quickly replaced. For this reasons the protection of restricted ecological zones, even with appropriate silvicultural management to ensure the survival of the species, might not be enough. Secondary genetic resources (*ex situ* conservation) are also necessary. Although at the moment *ex situ* programmes are mainly static, in the future it would be better to develop dynamic *ex situ* conservation.

Many European countries already started independent programmes of conservation of genetic resources of black poplar some years ago, mainly in consideration of its use as a parent species in poplar breeding. In recent years, however, the public concern about *P. nigra* as an endangered indigenous species has increased (Arbez 1993, White 1993). Unfortunately European countries generally lack exhaustive inventories of the remaining stands of *P. nigra* and detailed information on the status of preservation of the existing natural resources.

In situ conservation is limited to some particular situations: Bulgaria is involved in an international project for the protection of the riparian zones of the Danube. Croatia is going to start a plan for the preservation of the natural mixed forest with black poplar (covering about 10.000 ha). Hungary initiated a programme of gene preservation of native

species in 1992; at the moment 8 stands (about 60 ha) on private land are protected. Portugal created an 'exhibition stand' which won such recognition that a bill for the conservation of that species was issued. In Italy no protection systems exist for *in situ* preservation: some Regional Natural Parks or Natural Reserves have been created along the main rivers of Northern Italy which include areas with *P. nigra*, but no specific measure for the preservation of this species has been taken. The only indirect reference is some restriction on the cultivation of poplar hybrids. In France, the Network of Natural Riverside Reserves includes 1500 ha of riparian forest.

In contrast *ex situ* conservation is more common in Europe. In Italy the first collection of spontaneous *P. nigra* specimens started in central Italy before 1980, and was supplemented in 1981-83 by a large collection covering the whole national territory. The distance from areas of large-scale poplar culture, old trees, safety distance between sampled trees and an even sex-ratio were the guidelines of sampling (Bisoffi & al. 1987). In 1988 a thorough survey of the distribution of spontaneous poplar in the Lombardy Region was financed by the Regional Government (Anonymous 1989, Malinverno 1992). About 400 genotypes havee ben collected and planted in stool-beds, clonal banks and arboreta at the Istituto di Sperimentazione per la Pioppicoltura of Casale Monferrato.

In France, a first comprehensive collection started in the beginning of the 1970's in the upper valleys, where the risk of introgression from cultivated hybrids was considered lower, and it was continued later. 460 clones from 111 collecting sites are currently maintained in clonal banks and arboreta.

In Turkey about 500 clones were sampled at national level. Hungary started an inventory of *P. nigra* population some years ago: 150 clones propagated from isolated trees or from selected trees in native stands are now in their collection. Croatia, recently involved in a programme of conservation of black poplar, has selected and propagated 36 genotypes. In the UK an inventory of the existing *P. nigra* specimens and a collection of propagation material were recently started, with a press campaign that involved the public, strongly concerned about the conservation of native trees, in the survey.

In the Netherlands existing collections include about 80 clones, German collections include about 59 clones, while Spain has made a collection only in some restricted areas (about 110 clones). Bulgaria and Greece, instead, have only very small collections. Detailed information is not available for other countries.

As mentioned above, many countries are interested in *P. nigra* for its use in hybridization. Almost all the nations are now evaluating the variability of their collections as regards the characters useful for breeding: growth-related data (height and diameter), morphological traits (leaf shape, stem form and branchiness), phenology and resistance to principal diseases (*Marssonina, Melampsora, Dothichiza*) and insects (*Phloeomyzus passerinii* Sign.) are those considered.

Genetic diversity was also studied in France and Italy by means of biochemical markers (Legionnet & Lefevre 1996, Malvolti & Benedettelli 1993).

International coordination (EUFORGEN)

A project for the coordination of efforts aimed at the conservation of *P. nigra* has been recently launched as one of four pilot projects included in the European Forest Genetic Resources Programme (EUFORGEN) (IBPGR/FAO 1993, Arbez & Lefèvre 1996).

At the moment 12 countries have been involved: Austria, Belgium, Bulgaria, Croatia, France, Germany, Hungary, Italy, the Netherlands, Spain, Turkey and the United Kingdom. Network meetings were held in Izmit (Turkey) in 1994 (Frison & al. 1995) and in Italy in 1995 (Turok & al. 1996); a third one will be held in Hungary (1996). Present activities concern: the constitution of a European database for the *ex situ* collections, the establishment of a reference collection of *P. nigra* clones from 15 countries, the publiction of identification sheets and guidelines for the collection, the establishment of a list of standard descriptors, the survey of different *ex situ* and *in situ* solutions locally used for *P. nigra*, and literature reviews.

Proposals

Since *P. nigra* is a very valuable species for poplar breeders, a special effort must be made to maintain a large gene pool. Considering the widespread natural range of this species, particular attention must be devoted to preventing the loss of *P. nigra* resources in the marginal parts of its distribution area where selection may have favoured particular variants (local adaptations).

Further studies are necessary in order to get a better knowledge of the remaining wildstands of *P. nigra*, the biology of the species, and the structuring of genetic diversity. Such information may help, in the short run, for the definition of sampling for *ex situ* conservation. However, this kind of static conservation is not enough and, in the long run, it would be necessary to consider a form of 'dynamic' *ex situ* conservation, favouring flowering, crossing and recombination between genotypes in order to cope 'naturally' with changes of environmental conditions, including parasitic pressure – which is rapidly changing due to specific interactions with clonal poplar cultivars – or global climatic change.

The definition of an efficient strategy for 'dynamic conservation' (recombination and selection) of such a pioneer species, growing naturally in disturbed ecosystems, needs further research.

Acknowledgements

The contribution of information by many colleagues is gratefully acknowledged: Nuria Alba (Madrid, Spain); Stefano Bisoffi (Casale M.to, Italy); Sven DeVries (Wageningen, The Netherlands); Jòszef Gergacz (Sarvar, Hungary); Paul Koukos (Thessaloniki, Greece); Ante Krstinic (Zagreb, Croatia); Boudewijn Michiels (Geraardsbergen, Belgium); Antonio Padró Simarro (Zaragoza, Spain); Victor Steenackers (Geraardsbergen, Belgium); Tsanko Tsanov (Svischtov, Bulgaria); Korhan Tunçtaner (Izmit, Turkey).

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