

Genetic inventory of black poplar populations in the Upper Rhine floodplains: conclusions for conservation of an endangered plant species.

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Introduction

History

The development of riparian ecosystems along the Upper Rhine has gained a lot of interest during the course of centuries. As documented in ancient maps there was a meandering stream forming slopes, small islands and large riverbanks. There was also agricultural/arable land protected against floods by dams. In the floodplain area with frequently moving riverbanks we could have seen a softwood coppice forest with black poplars, willows and gray alder. Black poplar wood at small dimensions was used for heating throughout centuries. Shoots of poplars and willows were used to form bundles which could stabilize dams (fascine-work). In former times it was much more common to stabilize dams with fascine-work than it is nowadays because there was no machinery to transport heavy stone material. By the way of using such fascine-work, cloned material (stem sprouts) might have been distributed downstream and upstream. In contrast to basket willows, the cloning and distribution of certain varieties was not common for black poplars except for the group of fastigate-growing ‘Italica’-type of *P. nigra*. There are copper plates prepared about 1800 which show such black poplars growing along streets and waterways at many places in Germany (Struss 1987).

With increasing human activities a major correction of the Rhine riverbed took place. It was planned by an engineer called Tulla and lasted from 1817 till 1840. Major part of the former islands were eliminated, the riverbed became narrower and much deeper than before. In turn, the ground water level in the surrounding forest land was sinking. The ecological conditions did no longer support the softwood coppice forest with willows and black poplars. This caused dramatic changes in the composition of the vegetation as described by Volk (1998 see references). During the course of a century the coppice forest turned into a high forest with hardwoods (oak, common ash, elm, hornbeam, maples) and shrubs. In 1937 there was an equal area of planted hybrid poplars and autochthonous black poplars at the upper Rhine. After end of the war in 1948 it was most common to plant hybrid poplars but many more tree species were planted in former times. Nowadays within this ecosystem the ashes and maples show more abundant regeneration than black poplars.

Inventories

The first inventories of black poplars along the Upper Rhine were finished in 1996 (Franke *et al.* 1997). By means of morphological criteria like tree shape, stem form, bark and leaf characters our colleagues from the Federal State Baden-Württemberg identified overall 1124 adult trees in 8 forest districts along the Upper Rhine at a distance from Freiburg to Mannheim of ca. 180 km. Black poplars were dominating within forest communities if they were growing at stands with poor soils and higher ground water level where the conditions for ash, oak and elm trees were suboptimal.

Also in Hesse an inventory of black poplars confirmed the existence of true *P. nigra* stands in different forest districts along the Rhine river. A high number of trees were recorded and the identification by means of morphology was confirmed with isozyme analysis (Tab. 1).

Table 1: Black poplars in Hessian forest districts along the Upper Rhine. Confirmation of identification by isozyme analysis, see Janßen (1997), Janßen and Walter (1997). Source of data: Arbeitslisten 1997 / 1998 Az. Q10-02.01, HLFWW

Forest district	No. of adult black poplar trees	
	pure <i>P. nigra</i>	<i>P. x euramericana</i>
Lampertheim	29	0
Bensheim	433	6
Groß-Gerau	75	2
Chausseehaus	73	4
Eltville	76	2
Rüdesheim	17	1
Sum	703	15 (2,1 %)

In a study preceding the Europop project Janßen (1998) investigated 246 members of a natural regeneration of *P. nigra* L. at the Kühkopf/Knoblochsaue (population 1, see below). Only 2 individuals with heterozygote alleles specific to *P. x euramericana* were identified while 7 individuals revealed the pattern of F2 hybrids and 4 individuals could not be addressed to any group. With partners of the European research project “Genetic diversity in river populations of European Black Poplar for evaluation of biodiversity, conservation strategies, nature development and genetic improvement” two model populations at the Upper Rhine were chosen for further detailed studies.

Material and methods

Selection of populations

Population No. 1 is located in the nature protected area Kühkopf/Knoblochsaue (FA Groß-Gerau, Hessen), ca. 20 km south of Mainz. It comprises of 2300 ha with 60 % forest land. The so called Kühkopf area is an island formed by a slope of the river. Adult, reproducing trees are dispersed over the whole area. For flowering studies 64 adult trees were selected and mapped. The interior of the island is protected by a dam but in 1983 the dam was broken and in turn after this single flooding event at several sites a new generation of black poplars was established, see Janßen (1998).

Population No. 2 is located ca. 20 km south of Strasbourg within the so called Polder Altenheim (FA Kehl, Baden-Württemberg). This area is just downriver the plan d' eau de Plobsheim, a segment of the Rhine which is under intensive management. The polder and drainage was completed in the seventies. It comprises of almost 800 ha with 50 % of water bodies respectively small floodplain lakes. They have been produced by extraction of clay, sand and gravel. The whole area could enhance the flood discharge capacity of the river bed but without flood events these water bodies would be stagnant. Due to the transfer of soil in connection with polder construction, poplar regeneration was successful on former construction sites with suitable soil conditions. There are more than 120 old black poplars within the polder area.

Collection of samples

For studies on the leaf morphology and allozyme variation 30 individuals belonging to an adult and a nonreproductive generation of each population wer chosen. In addition six half sib families were raised from seeds collected from trees in population No. 1. Also ramets of clones belonging to different species within the section Aigeiros and clones from the German genebank collection (Fig.1) at Hann. Münden (Weserkamp Vaake) were used.

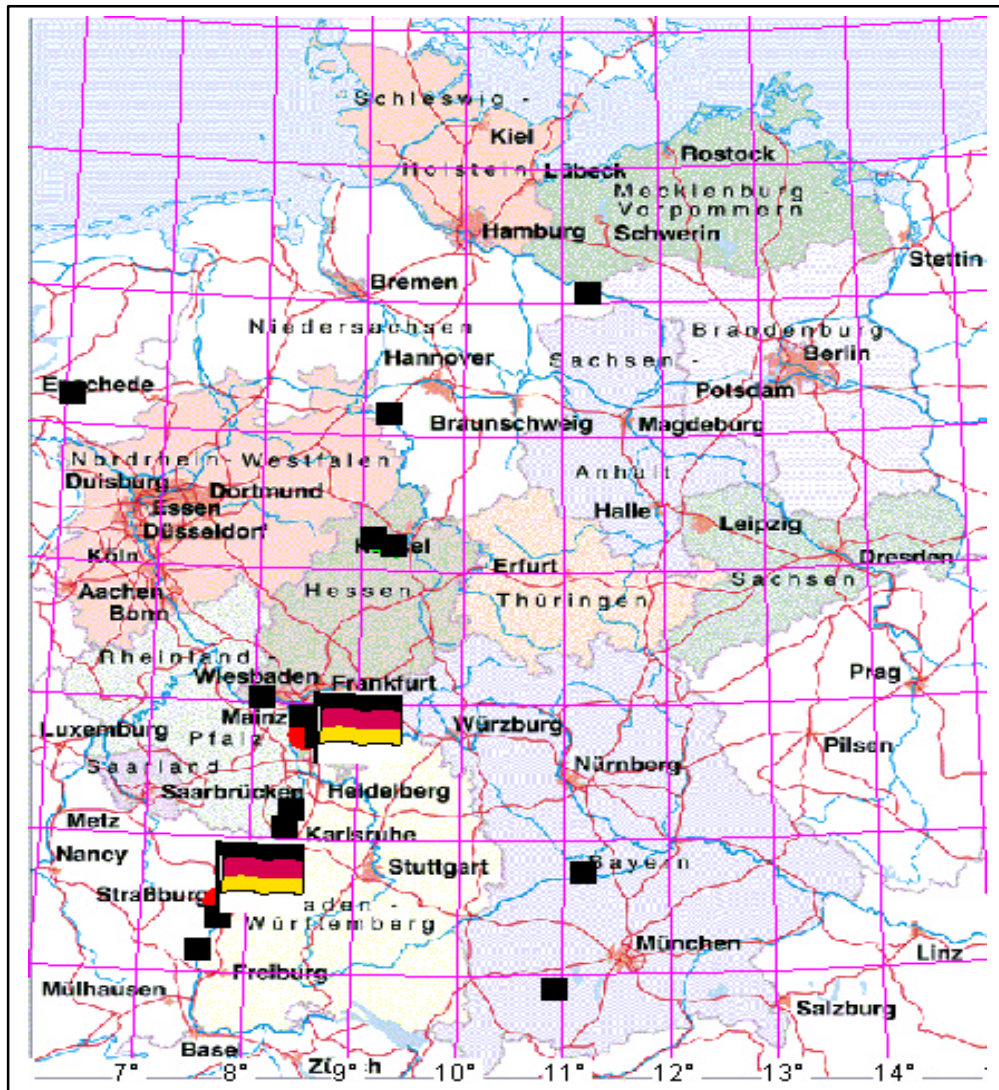


Figure 1: Origin of members of the German black poplar genebank in Hann. Münden (black squares) and location of the selected populations (red dots, flags).

Evaluation of leaf morphological characters

Regularly six leaves from six ramets per clone were subjected to measurements of leaf lengths (lengths of midrib in mm), maximum widths and petiole lengths. In addition the hairiness of the lower side (1, absent or very weak; 2, on the veins only; 3, on the whole leaf blade) and its intensity (1, weak; 2, medium; 3, strong) were scored. Also, the general shape of the leaf base

(base_shp: 1-11), the shape of the junction blade/petiole (junct: 1-6) and the shape of the tip (tip: 1-9) were scored according to a scheme derived by Jos van Slycken (Euforgen Plant Descriptors, 1996, ISBN 92-9043-272-1). In addition, the ratio of midrib length to maximum width (mid_wid) and the ratio of petiole length to midrib length (pet_mid) were calculated.

Evaluation of allozyme and microsatellite pattern

Electrophoretic separation techniques for allozymes followed the methods described by Konnert and Maurer (1995) for Norway Spruce. The detection and the evaluation of allozyme pattern were further standardized among the partners of the Europop project. The detection and evaluation of microsatellites refers to Van der Schoot *et al.* (2000). For statistical analysis all data were subjected to either Popgene, a program developed by Yeh *et al.* (1997) or the GSED program developed by Gillet (1994) which uses genetic distance measures of Gregorius (1984).

Results

Flowering of black and hybrid poplars

As demonstrated in Figure 2 the flowering of black and hybrid poplars in the area Knoblochsau was observed from 21st of March to 27th of April 2000. The scoring ranged from 0=dormant to 6=senescence of male flowers or fruit development, respectively.

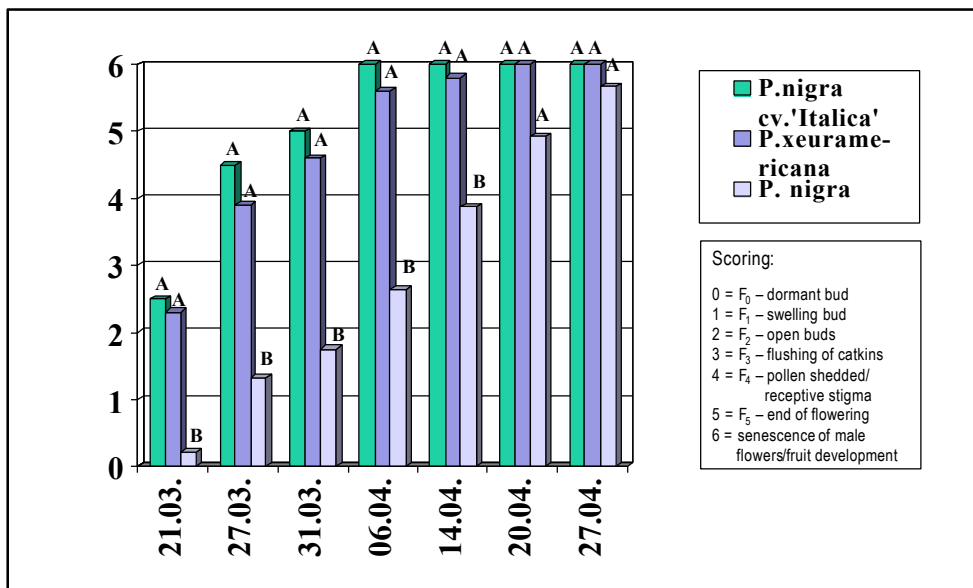


Figure 2: Flowering of black and hybrid poplars at the selected site (Knoblochsau).

Mean values of the flowering stages are shown. Individual scores were subjected to statistical analysis (GLM, Manova). Means followed by the same letter are not significantly different ($P=0,05$, REGWF-test).

It was obvious that hybrid poplars (*P. x euramericana*) and individuals of *P. nigra* 'Italica' flowered significantly earlier and hence, were scored higher than pure *P. nigra* until the 20th of april. When the stigmas of *P. nigra* became receptive, there was no more pollen shed from the hybrid poplars and neither from the variety *P. nigra* 'Italica' in this flowering season. In

addition the flowering data of 34 male and 23 female black poplars were subjected to statistical analysis. Male trees were scored significantly higher throughout the whole observation period. This indicates the phenomena of protandry which is common in many forest tree species.

Leaf morphology

In order to evaluate the leaf morphological characters from 454 individuals of the gene bank and reference collections including different species, selected cultivars of *P. nigra* (Nigra cvs) and wild types (nigra), all data were subjected to a stepdisc procedure (SAS). The method for selecting variables was stepwise with a significance level to enter/stay of 0.15. The variables hairiness and intensity of hairs turned out to be invariant. Therefore, they were excluded from further analysis. As demonstrated in Table 2, the lengths of the midrib followed by the lengths of the petiole were the most informative characters for species discrimination.

Table 2: Results of the stepdisc procedure, stepwise selection summary.

[abbreviations: lengths of midrib – midrib; lengths of petiole – petiole; relation midrib lengths/leaf widths – mid_wid; relation petiole lengths/ lengths of petiole – pet_mid; classification of base shape – Base_sh; leaf widths – widths; classification of junction – junct]

Step	Number In	Entered	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda
1	1	midrib	0.5424	75.52	<.0001	0.45759531	<.0001
2	2	petiole	0.5186	68.48	<.0001	0.22029711	<.0001
3	3	mid_wid	0.2099	16.85	<.0001	0.17406561	<.0001
4	4	pet_mid	0.2080	16.62	<.0001	0.13785510	<.0001
5	5	Base_sh	0.0954	6.66	<.0001	0.12470308	<.0001
6	6	width	0.0813	5.57	<.0001	0.11456739	<.0001
7	7	junct	0.0321	2.08	0.0441	0.11089279	<.0001

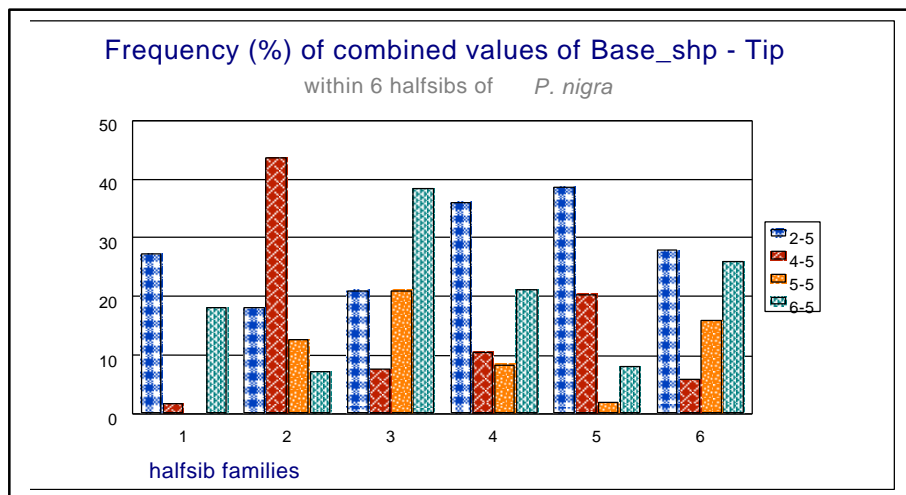


Figure 3: Frequency of combined values of base shape and tip form of leaves of 308 individuals from six half-sib families of *P. nigra* L.

Distribution of morphological characters within half-sib families

Leaves from 305 members of six half-sib families were scored and data were subjected to a frequency analysis procedure (SAS). As demonstrated in Figure 3, it became obvious that combinations of the leaf characters base shape (Base_shp) and tip form (Tip) occurred in different frequencies within different half-sib families. For instance, the combination of base shape 4 (broadly wedge-shaped) and tip 5 (broad long acuminate) was most frequent in half-sib family 2 and least frequent in half-sib family 1 (Fig.3).

Quantitative differences between populations and age classes

When the data from ramets of 24 juvenile (KK_NR) and 29 adult (KK_old) clones of population 1 (Kühkopf) together with the data from 12 juvenile (Po_NR) and 13 adult (Po_old) clones of population 2 (Altenheim polder) were subjected to a multiple analysis of variance (Manova, SAS), it became obvious that quantitative differences between populations and age classes are significant at the 5 % level of a REGWQ-test. The results of the tests are demonstrated in Figure 4.

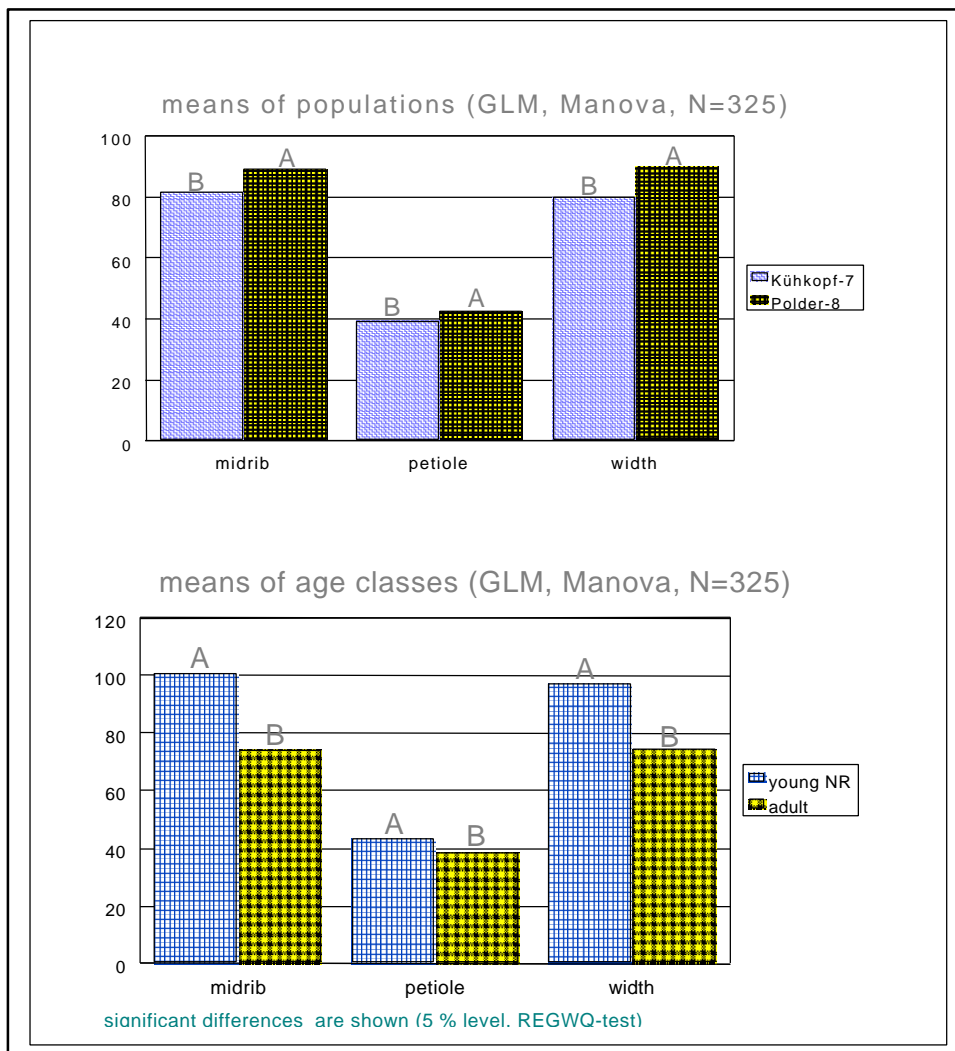


Figure 4: Differences between means of leaf characters of ramets of clones of two age classes selected from the two populations of *P. nigra*. Means marked by different letters are significantly different at the 5 % level (Manova, REGWQ-Test).

Genetic variation of allozymes

From a total of 11 analysed allozyme loci only 5 proved to be polymorphic. Common alleles with frequencies above 0.05 and rare alleles with frequencies below 0.01 occurred, see Table 3. Within the half sib families analysed we noticed deficiency of certain alleles but also an additional rare allele of IDH-A (allele 3) occurred within one half sib family.

Table 3: Allele Frequencies in the selected German populations and in the German genebank of *P. nigra* L (GBnigra), see M&M.

Locus/Allele	1/1-KK_NV	1/2-KK_old	2/1-Po_NV	2/2-Po_old	GBnigra
IDH-A, A1	0,4821	0,8548	0,6923	0,7143	0,5962
IDH-A, A2	0,5179	0,1452	0,3077	0,2857	0,4038
MDH-A, A1	0,9286	0,9062	0,9667	0,9667	0,9091
MDH-A, A2	0,0714	0,0938	0,0333	0,0333	0,0909
MDH-D, A1	0,8929	0,9062	0,9667	0,9667	0,9242
MDH-D, A2	0,1071	0,0938	0,0333	0,0333	0,0758
PGI-B, A1	0,9464	1,0000	1,0000	1,0000	0,9848
PGI-B, A2	0,0179	*****	*****	*****	0,0152
PGI-B, A4	0,0357	*****	*****	*****	*****
PGM-A, A1	0,9107	1,0000	0,9167	0,9167	0,9394
PGM-A, A2	0,0536	*****	0,0833	0,0833	0,0606
PGM-A, A3	0,0176	*****	*****	*****	*****
PGM-A, A4	0,0176	*****	*****	*****	*****

The summary of the F-statistics showed the effect of population subdivision (Fst), see Table 4.

Table 4: Summary of F-statistics and gene flow of allozyme loci [See Nei (1987) Molecular Evolutionary Genetics (p. 159-164)]

Locus	Sample Size	Fis	Fit	Fst	Nm*
IDH-A	278	0.1842	0.2411	0.0697	3.3344
LAP-A	306	****	****	0.0000	****
MNR-A	306	****	****	0.0000	****
MDH-A	306	-0.0817	-0.0690	0.0117	21.0497
MDH-B	306	****	****	0.0000	****
MDH-C	306	****	****	0.0000	****
MDH-D	306	0.0238	0.0380	0.0146	16.9156
6PGDH-A	306	****	****	0.0000	****
PGI-A	306	****	****	0.0000	****
PGI-B	306	0.2323	0.2521	0.0258	9.4501
PGM-A	306	0.0951	0.1113	0.0178	13.7557
Mean	303	0.1072	0.1466	0.0441	5.4169

* Nm = Gene flow estimated from $F_{st} = 0.25(1 - F_{st})/F_{st}$.

Nei's measures of identity and distance revealed a striking difference between the young (1/1) and old (1/2) generation in population No. 1, see Tab. 5.

Table 5: Nei's (1972) Original Measures of Genetic Identity and Genetic distance (below diagonal).

pop ID		1/2	1/1	2/1	2/2	GBn
1/2	adult individuals Pop1	****	0.9858	0.9961	0.9967	0.9932
1/1	young individuals Pop1	0.0143	****	0.9948	0.9939	0.9984
2/1	young individuals Pop2	0.0039	0.0052	****	1.0000	0.9986
2/2	adult individuals Pop2	0.0033	0.0061	0.0000	****	0.9981
GBn	members of genebank	0.0068	0.0016	0.0014	0.0019	****

Evaluation of microsatellite (SSR) data

The data of an analysis of 4 microsatellite data (S20, S16, S14, GC14) provided by P120 revealed also a low amount of subpopulation differentiation ($F_{st} = 0.0526$). In addition the young and old generation of population 1 was much more different than in population 2. The highest number of alleles was present in the genebank collection (Fig 5).

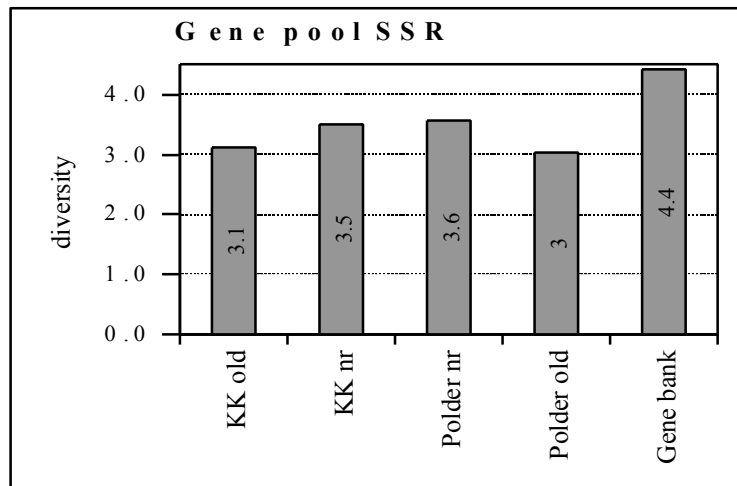


Fig. 5: Genetic diversity of the German populations and genebank, based on GSED (Gillet, 1984).

Allel F of the SSR locus PMGC14 was detected in the genebank collective and in population 2, but absent in population 1(Fig. 6).

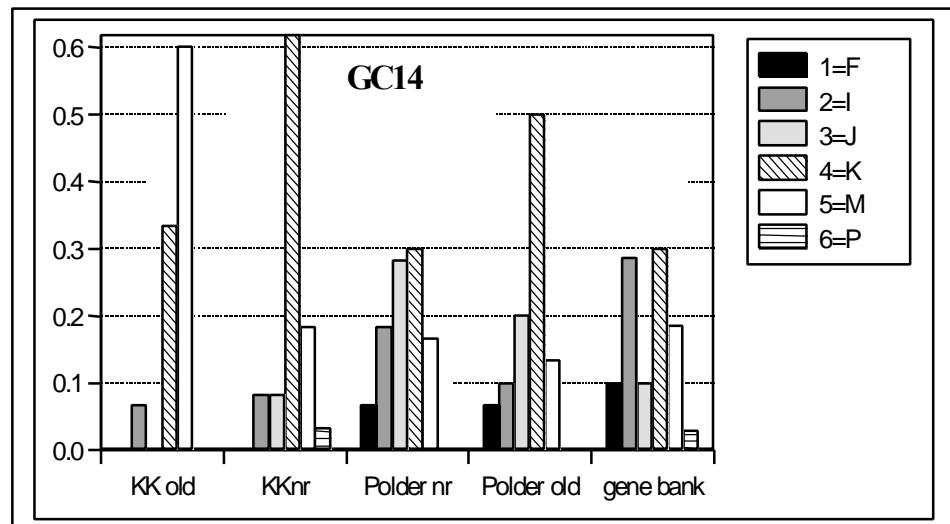


Fig. 6: Allel frequencies of the SSSR-locus PMGC14 within different collectives.

Clone identification

With the microsatellite data of P120 and the AFLP data provided by P 210 (Bart Ivens) it was possible to detect cloned material in population 1. Altogether 7 ramets of a single clone were detected by comparison of the 8 alleles of four microsatellite loci (PMGC14: MM; WPMS14: MO; WPMS16: EH; WPMS20: FG) and 53 AFLP alleles which proved to be identical. These ramets belong to the older generation of population No. 1. where the formation of root suckers was frequently noticed in vicinity to older trees (Fig. 7).



Figure 7: Vegetative regeneration of *Populus nigra* L. at the site of population 1 (Knoblochsaue).

Discussion

A genetic inventory of black poplars was based on allele frequencies of codominantly inherited isozyme and microsatellite loci of individuals from two populations separated at a distance of 170 km. *P. x euramericana* were identified by presence or absence of species specific alleles.

The analysis of leaf morphological data allowed the discrimination of poplar species and revealed significant differences between the two populations and trees belonging to a younger or older generation but different frequencies of leaf-morphological characters within the different halfsib families indicate maternal effects or dominance variance. Therefore, these characters cannot be considered to be neutral traits.

In previous studies, the difficulties involved in comparisons between qualitative traits such as isozymes or microsatellites and metric traits such as growth characters were pointed out (Lewontin 1984 and Felsenstein 1986).

Analysis of the flowering phenology revealed little risk of backcrosses of *P. nigra* with *P. x euramericana* or with the fastigate growing Italica-types of *P. nigra* in nature.

A comparison of the genetic diversity between different collectives of the two populations was based on allozyme variation. Occurrence of new rare alleles in half-sib families and differences between the young and adult generation within populations revealed the significance of gene flow mediated by pollen flight and seed dispersal. The amount of population differentiation was low ($F_{st} = 4\%$) but does not differ much from that of species with similar life history character. Different authors like Weber and Stettler 1981; Huyn *et al.* 1986; Jelinski and Cheliak 1992; reported F_{ST} -values of 1 to 7% in Salicaceae.

The evaluation of microsatellite (SSR) data revealed stand specific alleles and allele frequencies. This would in turn offer some sort of control for the distribution of germplasm. In some European countries forest managers are not allowed to distribute black poplar germplasm if this material is not autochthonous or tested and approved for trade. Therefore an effective way of control will be required.

Microsatellite and AFLP-data allowed the identification of unknown clones within the populations. A comparison of the genetic diversity between different collectives of the two populations, half-sib families and members of the German gene bank revealed the significance of gene flow mediated by pollen flight or large distance seed dispersal.

The present ecological situation of black poplars in the investigated stands is determined by the deep ground water level and the dominance of more shade tolerant tree species like common ash, hornbeam, alder, acer and hybrid poplars. Recent extreme floods in 1993 and 1995 have stressed the importance of floodplain restoration as described by Jürging (1996) and Prinz (1996). Rehabilitation of floodplain ecosystems should focus on genetic diversity of black poplars. The evolution of genetic diversity should be monitored in ecosystems. Measures of genetic diversity will indicate the balance of the mating system. Therefore recommendations can be given in order to improve genetic diversity within a stand. With the knowledge of the existing genetic diversity the needs for conservation of characters of subpopulations will be defined.

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Acknowledgements

We are grateful to Oberforstrat A. Franke (Forstl. Versuchs- und Forschungsanstalt Baden-Württemberg, Freiburg) for the provision of plant material. We would like to thank Dr. H. Volk (Forstl. Versuchs- und Forschungsanstalt Baden-Württemberg, Freiburg) for valuable informations about history and ecology of the Upper Rhine floodplains.