GENETIC DIVERSITY OF A BLACK POPLAR POPULATION IN THE MORAVA RIVER BASIN ASSESSED BY MICROSATELLITE ANALYSIS[1]

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ABSTRACT

Black poplar (*Populus nigra* L.) is an important pioneer tree species of riparian ecosystems, but it is endangered due to loss of its natural habitats in the west and central Europe at present.

The genetic structure of the population from the Morava river basin in the Czech Republic was investigated by microsatellite analysis. 112 adult trees (50–140 years old) were characterised at 12 microsatellite loci.

There were identified 8 groups of identical genotypes (altogether 24 trees) among these individuals. All tested loci were highly polymorphic with a mean number of alleles 13.4 per locus, but almost 17 % of alleles were rare, appearing only once. The high values of expected heterozygosity and effective number of alleles (0.83 and 6.5, respectively) indicate high diversity in the population as well as the high value of observed heterozygosity (0.79) shows large number of heterozygous individuals. The positive mean of single-locus fixation index values indicates a weak overall excess of homozygotes.

In order to study the spatial structure and gene flow within the population, four individual study sites were considered as subpopulations. Low F_{ST} values confirm the existence of gene flow among study sites and small genetic differentiation among them. However the calculated variation parameters could be loaded by considerable sampling error, since the numbers of trees in the individual study sites were rather small.

Key words: *Populus nigra*, DNA polymorphism, genetic diversity

INTRODUCTION

The black poplar (*Populus nigra* L.) is a dioecious tree species with broad geographical distribution ranging from western and southern Europe to central Asia and northern Africa. It is an important pioneer component of riparian forests, where it colonizes open areas near streams since it is strictly heliophilous and hydrophilous. Pollen and seeds are dispersed by wind (seeds also by water) over considerable distances.

Unfortunately, large areas of its natural habitat mostly in western and central Europe have been lost due to drainage of rivers, management of riverbanks and wood cutting. Black poplar's capability for natural regeneration has been restricted in consequence of flood control. The knowledge of genetic diversity and population structure in remaining populations is a prerequisite for the successful management of conservation programs in future.

LEGIONNET and LEFÈVRE (1996) used isoenzymes to describe the level and organization of the genetic diversity in *Populus nigra* in France. They detected low overall diversity level and low differentiation at three geographical scales, although gene flow was limited.

ARENS *et al.* (1998) investigated the genetic structure of black poplar populations along the Dutch Rhine using the AFLP technique. They identified a lot of vegetatively propagated individuals and found high similarities among remaining trees. Also WINFIELD *et al.* (1998) used the AFLP procedure for monitoring of genetic diversity in black poplar in the Upper Severn area of the UK and found there a low level of it. When trees were physically close to each other, AFLP profiles were found to be very similar and often even identical. More results were obtained in the study of genetic diversity of 23 black poplar populations along six European rivers, where authors compared different methods (morphological characteristics, isoenzymes, cpDNA, AFLP and microsatellites) (VAN DAM *et al.* 2002). The genetic diversity within populations and within river systems was high as well as the genetic

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differentiation between river systems and between populations within a river system. The authors also found out that although black poplar is capable of vegetative reproduction, this method is not sufficient effective for the natural colonization of new territories. On the other hand it plays an important role in areas with high level of human interference.

In this study, twelve microsatellite markers developed for poplar were used to assess the population structure and genetic variability in the Moravian black poplar population. Because distances among four particular study sites within our population could be important (the biggest distance between two of them was 39 km), we also attempted to verify the existence of more or less isolated study sites and considered each study site as an independent population in spite of the assumption of long-range pollen transport.

MATERIALS AND METHODS

One black poplar population, consisting of four study sites, was sampled in the Litovelské Pomoraví Protected Landscape Area and Zástudánčí Nature Reserve in the broad vicinity of the town Olomouc in central Moravia (49°36′N/17°15′E). Exact positions of study sites Mladeč, Střeň, Bystrovany and Zástudánčí and numbers of trees per study sites are given in Figure 1. In each study site all black poplar trees, which were found, were also sampled. Young fresh leaves were collected from 112 adult trees (50–140 years old) and stored at +4 °C until the DNA extraction.

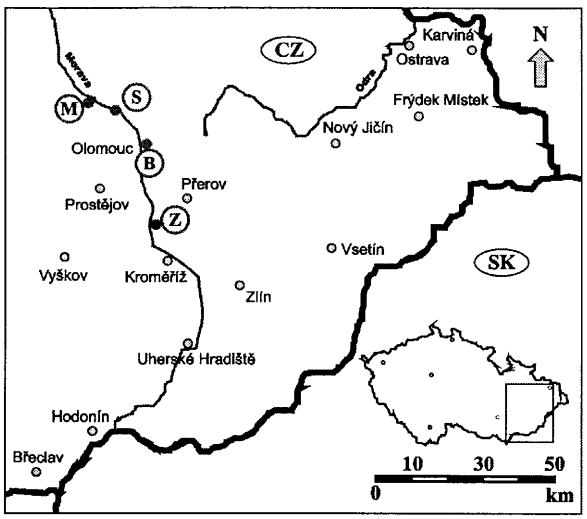

Figure 1. Locations of four investigated black poplar study sites: M – Mladeč (13 trees), S – Střeň (42 trees), B – Bystrovany (31 trees), Z – Zástudánčí (15 trees).

Total DNA was extracted from single leaves using the Dneasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Among the nuclear micro-satellites described for poplar by VAN DER SCHOOT *et al.* (2000) and SMULDERS *et al.* (2001) and listed in Poplar Molecular Genetics Cooperative SSR Database (previously http://poplar2.cfr.washington.edu/pmgc, but it does not exist anymore) twelve polymorphic loci with good quality of banding patterns were chosen: WPMS03, WPMS04, WPMS05, WPMS07, WPMS09, WPMS11, WPMS12, WPMS14, WPMS16, WPMS18, WPMS20 and PMGC14. Amplification reactions and electro-phoretic separation of products followed VAN DER SCHOOT *et al.* (2000). For the PMGC14 from the PMGC database, the primers were
5′-TTCAGAATGTGCATGATGG-3′ and
5′-GTGATGATCTCACCGTTTG-3′ and the PCR was performed in the same way as for WPMS12.

The products were visualised by silver staining according to the Promega Silver Sequence DNA Sequencing System. The sizes of the PCR products were determined by comparison to an accompanying sequence reaction using pGEM®-3Zf(+) control DNA (Promega).

Basic characteristics of genetic variability were determined: allele frequencies, observed (H_o) and expected (H_e) heterozygosity (NEI 1973), mean number of alleles per locus (A) and mean effective number of alleles per locus (n_e). For each locus, deviation from Hardy-Weinberg expectation was examined by calculating Wright's fixation index as $F_{is} = 1 - (H_o/H_e)$. The population F_{is} values were estimated as the mean of F_{is} values over all loci. The degree of differentiation between study sites was estimated with Wright's index of population subdivision F_{st} (WEIR 1990), and genetic divergence between study sites was estimated by Nei's genetic distances (NEI 1978).

These analyses were carried out with POPGENE 1.31 (YEH *et al.* 1999) and GENEPOP 3.4 (RAYMOND & ROUSSET 1995). The relationship between Nei's genetic distance and geographical distance in km was examined with UNISTAT 4.53 by estimating the Spearman rank correlation coefficient.

RESULTS

Genetic diversity in the global population

All twelve analysed microsatellite loci were highly polymorphic with the least variable WPMS20 locus and the most variable WPMS04 locus displaying 6

Table 1. Allelic frequencies for nine microsatellite loci in the global black poplar population. Rare alleles with frequencies lower than 0.01 are typed in italic.

Allele	Locus											
	WPMS 03	WPMS 04	WPMS 05	WPMS 07	WPMS 09	WPMS 11	WPMS 12	WPMS 14	WPMS 16	WPMS 18	WPMS 20	PMGC 14
A	0.0417	0.0312	0.1198	0.0208	0.0990	0.0052	0.0208	0.2240	0.3542	0.0156	0.1823	0.2448
B	0.0208	0.0417	0.0052	0.0052	0.0573	0.0104	0.5365	0.0104	0.0729	0.1823	0.1615	0.1615
C	0.0938	0.1198	0.0104	0.0052	0.0052	0.0260	0.1667	0.1302	0.1250	0.1615	0.1979	0.1927
D	0.1354	0.2500	0.0260	0.1667	0.3333	0.1458	0.1562	0.2708	0.2292	0.2552	0.1771	0.1615
E	0.0781	0.0469	0.1719	0.0052	0.1042	0.0833	0.0208	0.0469	0.0312	0.0104	0.1823	0.1198
F	0.1042	0.0677	0.0156	0.0052	0.0312	0.0208	0.0469	0.0625	0.1823	0.0833	0.0990	0.0260
G	0.0625	0.0052	0.1562	0.0469	0.1927	0.1198	0.0417	0.0625	0.0052	0.0677		0.0052
H	0.0365	0.0052	0.0677	0.0156	0.0469	0.0052	0.0104	0.0104		0.1615		0.0885
I	0.3281	0.0104	0.0729	0.0312	0.0729	0.0052		0.0052		0.0208		
J	0.0521	0.0052	0.0417	0.0312	0.0521	0.0052		0.1250		0.0260		
K	0.0208	0.0208	0.1875	0.0104	0.0052	0.0573		0.0052		0.0156		
L	0.0104	0.0052	0.0260	0.0312		0.0469		0.0260				
M	0.0052	0.0052	0.0104	0.0833		0.0260		0.0208				
N	0.0052	0.0312	0.0052	0.0938		0.0573						
O	0.0052	0.0104	0.0833	0.2240		0.1927						
P		0.0417		0.1667		0.0729						
Q		0.0677		0.0208		0.0417						
R		0.0260		0.0365		0.0052						
S		0.0365				0.0104						
T		0.0208				0.0469						
U		0.0052				0.0156						
V		0.0417										
W		0.0312										
X		0.0052										
Y		0.0312										
Z		0.0156										
a		0.0156										
b		0.0052										

and 28 alleles, respectively. Owing to very different degree of polymorphism for single loci, values of average number of alleles per locus (13.42) and average effective number of alleles per locus (6.51) showed generally high standard deviations. Almost 17 % of observed alleles were rare, appearing only once in the population (Table 1).

After detection of allele combinations, eight groups of identical genotypes were identified (altogether 24 trees). These ramets are probably artificially vegetatively propagated as each group occurs always in the same study site usually near inhabited areas. Therefore they were excluded from the next analyses except of one randomly selected individual from each group. So in the end we evaluated 96 trees in all.

Mean expected and observed heterozygosity for single loci ranged in the global population from 0.665 to 0.899 and from 0.667 to 0.927, respectively, indicating high variability. The positive mean of single locus fixation index values (0.041) indicates a weak overall excess of homozygotes (Table 2). F_{is} values for single loci showed that a surplus of homozygotes existed for seven loci (WPMS03, WPMS05, WPMS07, WPMS11, WPMS14, WPMS16 and WPMS18), but only three of these loci (WPMS05, WPMS11 and WPMS18) showed significant deviations from zero ($P < 0.01$).

Genetic analysis of four study sites

First of all it is necessary to remark small numbers of individuals in study sites, which could affect calculated parameters. Across study sites, expected and observed heterozygosities were high, indicating high variability within study sites. The average intrapopulation fixation index F_{is} was low (–0.0152). Two of our study sites (Mladeč and Zástudánčí) showed an overall significant departure from Hardy-Weiberg genotypic proportion with fixation coefficients F_{is} = –0.066 and F_{is} = –0.079, respectively (Table 2), indicating an excess of heterozygotes.

The values of F_{st} that can be interpreted as the

Table 2. Characteristics of the genetic multiplicity and diversity of the black poplar populations[a]. Values in parentheses are standard deviations.

Population	N	A	n_e	H_o	H_e	F_{is}
Global population	96	13.4 (6.5)	6.51 (2.09)	0.793 (0.076)	0.829 (0.066)	0.041**
Mladeč	13	7.3 (2.4)	4.87 (1.52)	0.821 (0.068)	0.775 (0.072)	–0.066
Střeň	40	10.4 (4.2)	5.99 (1.69)	0.783 (0.092)	0.817 (0.065)	0.041**
Bystrovany	20	8.4 (2.0)	4.76 (1.51)	0.742 (0.126)	0.772 (0.065)	0.042*
Zástudánčí	23	8.5 (3.5)	5.06 (1.58)	0.841 (0.102)	0.781 (0.086)	–0.079

[a] N – mean sample size per locus; A – mean number of alleles per locus; n_e – mean effective number of alleles per locus; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{is} – fixation index.

Table 3. Single-locus F-statistics in the four investigated black poplar study sites. Values in parentheses are standard deviations.

Locus	F_{is}	F_{it}	F_{st}	N_m*
WPMS03	0.0954	0.1303	0.0386	6.2336
WPMS04	–0.0698	–0.0300	0.0372	6.4750
WPMS05	–0.0581	0.0104	0.0647	3.6124
WPMS07	0.0405	0.0733	0.0342	7.0655
WPMS09	–0.0510	–0.0115	0.0377	6.3899
WPMS11	0.1216	0.1809	0.0675	3.4537
WPMS12	–0.0973	–0.0647	0.0297	8.1621
WPMS14	–0.0405	0.0164	0.0547	4.3190
WPMS16	0.0609	0.1021	0.0439	5.4463
WPMS18	–0.0367	0.0512	0.0847	2.7014
WPMS20	–0.0882	–0.0038	0.0776	2.9716
PMGC14	–0.0597	–0.0093	0.0476	5.0053
Mean	–0.0152 ± 0.0745	0.0371 ± 0.0721	0.0515 ± 0.0181	4.6044

* N_m: gene flow estimated from F_{st}: $N_m = 0.25\,(1 - F_{st}) / F_{st}$

Table 4. Nei's genetic distances (below diagonale) and identities (above diagonale) among four study sites in the investigated black poplar population.

Locality	Mladeč	Střeň	Bystrovany	Zástudánčí
Mladeč	–	0.779	0.648	0.722
Střeň	0.250	–	0.825	0.855
Bystrovany	0.434	0.192	–	0.789
Zástudánčí	0.326	0.156	0.237	–

proportion of genetic variation among study sites, ranged from 0.0297 to 0.0847 for single loci (Table 3). Mean F_{st} across all loci and study sites was 0.052. Thus, about 5 % of the total measured genetic variation in black poplar was attributable to variation among study sites. The value of *Nm*, the number of migrants between study sites per generation, was estimated to be about 4.6.

Values of Nei's genetic distances between pairs of study sites varied from 0.156 (Střeň – Zástudánčí) to 0.434 (Mladeč – Bystrovany) (Table 4). Spearman rank correlation coefficient ($r_s = -0.2571$, $P = 0.56$) did not show any correlation between genetic and geographical distances. Clustering among study sites using UPGMA did not reflect their geographical proximity (results not shown).

DISCUSSION

Our study discovered considerable proportion (about 21 %) of clones in the Moravian black poplar population. Vegetative propagation of poplar is feasible, but this method has real importance mainly in areas with high level of human intervention. For example WINFIELD *et al.* (1998) obtained very similar and usually identical AFLP profiles for samples of black poplar taken from closely located individuals in the Upper Severn area in UK. In author's opinion these results favour the hypothesis that black poplar has been maintained by cuttings in this area. Also our clones were concentrated (14 of 24 clones) in the study site Bystrovany, which is very closed to urban area.

This study revealed very high level of genetic diversity even in this limited population in comparison with studies based on isoenzyme markers. DAMM (2002) found low level of genetic diversity (H_e = 0.126, n_e = 1.18) with 8 isoenzymes for 23 black poplar population along 6 rivers across all Europe in the EUROPOP project. LEGIONNET and LEFÈVRE (1996) analysed black poplars from 111 sites in France by 8 isoenzyme markers and detected mean heterozygosity H = 0.198. HAMRICK and GODT (1989) stated mean heterozygosity based on isoenzymes for forest trees H = 0.15.

Our finding are in accordance with results of microsatellite analysis reported by VAN DAM *et al.* (2002) for three populations of black poplar along Rhine in Germany and in the Netherlands and with results reported by ALBA *et al.* (2002) for two populations in Spain. They found high values of expected heterozygosity (0.73 and 0.63, respectively) and for the populations along Rhine also high values of mean number of alleles per locus (8) and observed heterozygosity (0.68).

Surprisingly, our observed and expected heterozygosities and mean number of alleles per locus correspond also to those found by VAN DAM (2002) by means of microsatellite markers for 23 black poplar populations mentioned above (H_e = 0.79, H_o = 0.77 and A = 15.4). They used 7 nuclear microsatellites; all of them were used also in our study. The very high value of genetic diversity within poplar populations can be explained by the high degree of polymorphism of analysed microsatellite loci (since they are neutral markers), the fact that poplar as dioecious species is strictly outcrossing, and that pollen and seeds can be dispersed over large distance by wind (seeds also by water).

But also in studies of other deciduous temperate tree species using microsatellite markers, similarly high polymorphism has been reported. For example, STREIFF *et al.* (1998) detected mean number of alleles per locus 21.7 in *Quercus robur* and *Q. petraea*, values of observed and expected heterozygosity were estimated 0.81 and 0.87, respectively. Similar results are reported by HEUERTZ *et al.* (2001) and by MORAND *et al.* (2002) for *Fraxinus excelsior* (A = 12.4 and 9.6, H_e = 0.73 and 0.898, respectively) and by ODDOU-MURATORIO *et al.* (2001) for *Sorbus torminalis* (A = 10.7, H_e = 0.78, H_o = 0.77).

The observed positive mean of fixation index values indicates weak excess of homozygotes in the population. The positive F_{is} values in most populations suggest inbreeding, but only when all studied loci show equally high F_{is} values. High F_{is} values observed for a part of the studied loci indicate selection for these loci or existence of null alleles. An alternative explanation may be the Wahlund effect due to the presence of breeding subunits inside the studied population. For our population we can explain the positive F_{is} by means of null alleles in the WPMS11 and WPMS03 loci, which we found recently at the controlled crossing (unpublished results). The positive and high F_{is} values for these loci heavily influence the calculation of the average F_{is} across all loci. Null alleles of microsatellite regions, which show themselves by absence of an amplification product, may arise through a point mutation at one or other priming site.

The overall degree of population differentiation was small (F_{st} = 0.052) for four study sites inside the Moravian black poplar population. Thus, about 95 % of total genetic variation resides within each study site. Such low values also have been reported for other black poplar populations from relatively small regions. For two populations in Germany F_{st} was 0.053 (GEBHARDT *et al.* 2002) and for three populations along Rhine F_{st} was 0.05 (VAN DAM *et al.* 2002). On the contrary, the differentiation among 23 black poplar populations from all Europe was much higher (F_{st} = 0.314) (VAN DAM 2002). These results are accordant with the principle that maintenance of low variation among populations depends on the efficiency of gene flow, a factor that usually is supported by effective cross-pollination and seed dispersal mechanisms. The low genetic differentiation among our four study sites indicates that pollen and seed flow can reach large distances and prevent differentiation. This conclusion confirms our presumption that all analysed individuals probably represent one large intermating population. But herewith it is necessary to take into account that the numbers of trees in the individual study sites were rather small, and that statistical comparisons based on them could be loaded by considerable sampling error.

Nei's genetic distances between pairs of study sites reached high values. BORDÁCS *et al.* (2002)

reported the distances between three Hungarian black poplar populations varied from 0.0022 to 0.007 for isoenzymes and from 0.0176 to 0.05 for cpDNA markers. GEBHARDT *et al.* (2002) showed Nei's genetic distances 0.0033 based on isoenzymes for two German populations. Our extremely high values resulted probably from the insufficient number of trees in individual study sites and also from the high level of polymorphism of microsatellite markers.

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