Acta Societatis Botanicorum Poloniae

DOI: 10.5586/asbp.3524

Publication history
Received: 2016-04-29
Accepted: 2016-12-04
Published: 2016-12-30

Handling editor
Mirela Tulik, Faculty of Forestry, Warsaw University of Life Sciences – SGGW, Poland

Authors' contributions
AL designed the study; AL, ML carried out genetic analysis, contributed to the writing of the manuscript

Funding
The research study was supported by the Institute of Dendrology, Polish Academy of Science.

Competing interests
No competing interests have been declared.

Citation
Lewandowski A, Litkowiec M. Genetic structure of the old black poplar population along the bank of the Vistula River in Poland. Acta Soc Bot Pol. 2017;86(1):3524. https://doi.org/10.5586/asbp.3524

SHORT COMMUNICATION

Genetic structure of the old black poplar population along the bank of the Vistula River in Poland

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Abstract

Black poplar (*Populus nigra* L.) is one of the main woody riparian species in Europe. Because of extensive habitat loss due to river regulations, this species is considered rare and threatened. To analyze genetic diversity and spatial genetic structure, we examined ten nuclear microsatellite loci in a population of very old *P. nigra* trees growing along the Vistula River in Poland. We found a high level of genetic diversity (H_E = 0.792, H_O = 0.731, A = 14.7) that was within the range of other natural European *P. nigra* populations, and our results showed that sexual propagation is the dominant way of reproduction in the studied population, leading to high clonal diversity (R = 0.91). Additionally, we did not detect a spatial genetic structure resulting in a random spatial distribution of genotypes. Individuals from such old and diverse populations have the potential to provide valuable reproductive material for both restoration programs and breeding purposes.

Keywords

conservation; nuclear microsatellite markers; *Populus nigra*; riparian ecosystems

Introduction

Black poplar (*Populus nigra* L.) is a pioneer species of riparian woodlands, occurring as apparent metapopulations, and an indicator of the good condition and biodiversity of these ecosystems. However, due to long-term human impacts, particularly agricultural development and urbanization, riparian habitats have largely disappeared, and *P. nigra* is currently one of the most endangered tree species in Europe [1]. Therefore, projects for in situ conservation and restoration of black poplar have been initiated in many countries as part of European forest genetic resource programs [2]. The effectiveness of these programs depends on information about the level of genetic variation and genetic differentiation between populations of threatened species, and identifying regional gene pools of *P. nigra* is essential for the success of conservation actions. Conservation efforts should focus on maintaining large and more diverse populations that may be utilized as a source for restoration projects. *Populus nigra* also plays a key role as a parental pool in poplar breeding programs, and the species has contributed to the breeding of many successful interspecific hybrids [3].

The main objective of this study was to estimate the genetic diversity and spatial genetic structure of a natural population of very old *P. nigra* trees from Dęblin in Poland, located in the middle section of the Vistula River, using a set of nuclear microsatellite loci.

Fig. 1 *Populus nigra* trees in the studied population. (phot. A. Lewandowski).

Material and methods

The studied area is situated in the middle section of the Vistula River on the right bank near Dęblin, Poland (51°34′00″ N, 21°51′41″ E). The site is a remnant of the natural floodplain forests and is very strongly affected by human activities. For a long time, it was used as a military training area. Fifty-two putatively pure black poplar trees presumably more than 150 years old grow in the analyzed transect (approximately 10 ha) (Fig. 1). We mapped all trees in the area; for all standing trees, we also recorded the circuits of surveyed trees and sex. Leaves from the 52 individuals were sampled in spring of the 2014 year, and genomic DNA was extracted using a modified CTAB protocol [4].

We analyzed ten nuclear microsatellite (nSSRs) loci that were combined into two 5-plexes. The first multiplex consisted of WPMS02, WPMS03, WPMS05, WPMS10, and WPMS12; the second multiplex included WPMS01, WPMS04, WPMS06, WPMS07, and WPMS09. The fluorescently labeled PCR products, along with a size standard (GeneScan 500 LIZ), were separated using an ABI 3130 capillary sequencer (Life Technologies, USA). Allele identification based on size was determined using GeneMapper software (ver. 4.0; Life Technologies, USA) and confirmed manually.

The genetic diversity of the ten loci was estimated based on the following parameters, all of which were computed using FSTAT v 2.9.3 and GenAlEx 6 [5]: the total number of alleles (A), the effective number of alleles (A_E), the observed heterozygosity (H_O), and the unbiased expected heterozygosity (H_E). Deviation of genotypic frequency from Hardy–Weinberg equilibrium (HWE) was identified utilizing inbreeding coefficients (F_{IS}), for which significance levels were adjusted using the exact test based on the Markov chain Monte Carlo algorithm (MCMC) implemented in GENEPOP v. 4.0 [6]. Null alleles are typically present in SSR markers (e.g., [7]) and can overestimate the F-statistic in populations due to false homozygotes. Therefore, the frequency of null alleles (N_0) at each locus and the inbreeding coefficient F_{IS} with null allele correction ($F_{IS\,Null}$) were estimated based on the individual inbreeding model (IIM) with a Gibbs sampler with 105 iterations using INEST 1.0 software [8].

All calculations based on microsatellite data were performed using the GenClone Software package (v 2.0) [9]. The level of within-population clonal diversity was

estimated using genotypic richness (R), defined as $R = (G - 1)/(N - 1)$, where G is the number of genotypes and N is the number of samples. The correspondence of MLGs to real clones was tested using the GenClone estimate of the p_{sex} index, which is the probability that a given MLG could occur as the result of independent sexual reproduction events rather than as the result of clonal reproduction within a naturally reproducing population, according to Parks and Werth [10]. The intensity of spatial genetic structure (SGS) was evaluated by the Sp statistic [11,12].

Results and discussion

Surprisingly, despite its limited size, the studied population was found to be characterized by a high level of genetic variation (Tab. 1), which is in agreement with previous genetic studies of *Populus nigra* employing a variety of genetic marker systems [13–18]. All nSSR loci were highly polymorphic. The number of alleles ranged from 7 (WPMS02) to 25 (WPMS04), with a mean of 14.7, although the average effective number of alleles was much lower (3.7), ranging from 1.4 (WPMS02) to 11.1 (WPMS07). The level of observed heterozygosity (H_O = 0.731) was lower than the level of expected heterozygosity (H_E = 0.792). In addition, evidence of the presence of null alleles at the investigated loci was found, ranging from 2.4% (WPMS04) to 7.5% (WPMS03). The diversity parameters estimated in our study (H_E = 0.79 and A = 14.7) strongly correspond to those found by Pospiśková and Sàlkovà [14] for the *P. nigra* population along the Morava River (Czech Republic), close to Polish territory (H_E = 0.82, A = 11), as well as results obtained by van Dam [19] based on an analysis of 23 *P. nigra* populations along six rivers across Europe (H_E = 0.79, A = 15.4). Within the context of genetic conservation, it is particularly important that the population from Dęblin still maintains a high number of alleles per locus.

Because null alleles were detected in all loci, with a mean frequency reaching nearly 4.1% (Tab. 1), we applied the IIM approach to assess their influence on $F_{IS\ Null}$. We detected low $F_{IS\ Null}$ ranging from 0.021 for WPMS04 to 0.106 for WPMS03, with an overall mean of 0.046. The value of the inbreeding coefficient F_{IS} was nearly twice

Tab. 1 Parameters used to estimate the genetic structure of the studied *Populus nigra* population averaged across 10 nuclear microsatellite loci.

Locus	A	A_E	H_E	H_O	F_{IS}	PHW	$F_{IS\ Null}$	N_0 (%)
WPMSO1	19.0	10.0	0.900	0.846	0.060	0.0187	0.025	3.5
WPMSO2	7.0	1.4	0.297	0.269	0.094	0.0510	0.052	6.0
WPMSO3	15.0	7.5	0.866	0.654	0.245	<0.0010	0.106	7.5
WPMSO4	25.0	7.6	0.868	0.885	–0.019	0.1061	0.021	2.4
WPMSO5	9.0	5.3	0.810	0.788	0.026	0.3162	0.038	3.0
WPMSO6	20.0	10.3	0.903	0.788	0.127	0.0023	0.057	4.7
WPMSO7	16.0	11.1	0.910	0.885	0.028	0.2419	0.027	2.5
WPMSO9	12.0	6.9	0.857	0.808	0.058	0.0138	0.042	3.7
WPMSO10	16.0	10.3	0.903	0.788	0.127	0.0434	0.060	4.3
WPMSO12	8.0	2.6	0.609	0.596	0.021	0.4857	0.039	3.7
Average	14.7	3.7	0.792	0.731	0.078	<0.0010	0.046	4.1

A – average number of alleles; A_E – effective number of alleles; H_E – unbiased expected heterozygosity; H_O – observed heterozygosity; F_{IS} – inbreeding coefficient; PHW – the test of significance for Hardy–Weinberg equilibrium; $F_{IS\ Null}$ – inbreeding coefficient with correction of null allele frequency; N_0 (%) – null allele frequency.

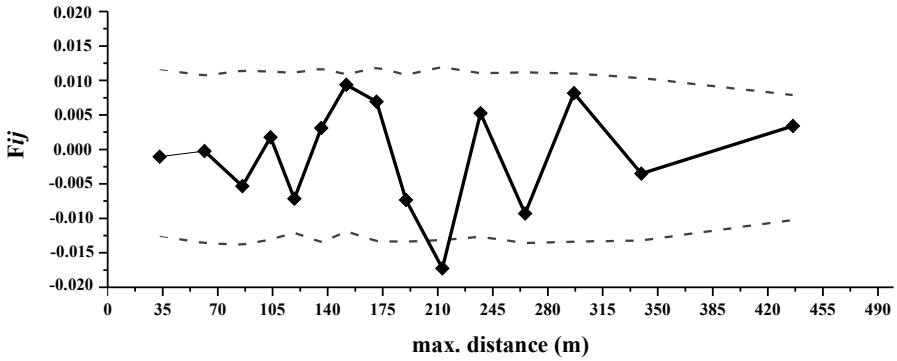

Fig. 2 Correlogram (solid line) of the spatial genetic structure of a *Populus nigra* population near Dęblin. Loiselle's kinship coefficient (F_{ij}) is plotted for eight distance classes with respective 95% confidence intervals (dotted lines).

that of $F_{IS\ Null}$, which showed an average of 0.078. Additionally, the F_{IS} value for a single locus reflected an excess of heterozygotes only for one locus (WPMS04). Statistically significant deviations from HWE were detected for five loci (Tab. 1). The observed positive value of the mean fixation index indicates a slight excess of homozygotes even after correction for the presence of null alleles. In contrast to our results, homozygote deficiency is often found in natural populations of *P. nigra* at mature stages [13,15,16].

Among the 52 sampled *P. nigra* trees, 21 were male and 15 were female, with a sex ratio of 1:0.71 (male excess). This ratio was not significantly different from a 1:1 ratio ($p > 0.01$). Due to the absence of flowers, the sex of 16 trees was undetermined. The mean circuit values for the male and female trees were similar at 518 (CI: 370–680) and 528 (370–710), respectively. The completed genotypes for all 10 loci were obtained for all 52 sampled individuals of *P. nigra*, with 47 unique MLGs identified. Genotypic richness was high ($R = 0.91$), with only 9.6% of the sampled trees being duplicates. The distance between two ramets within a clone ranged from 12 to 140 m. Our results showed that sexual propagation, which led to high clonal diversity ($R = 0.91$), is the dominant way of reproduction in the studied population. Most studies of *P. nigra*, including the present one, report a "phalanx" growth form, usually with a small number of ramets per clone. High levels of clonal diversity in black poplar were previously reported by Legionnet et al. [20] in six natural stands located in different regions of France. However, others have indicated that asexual regeneration strategies play an important role in the maintenance of *P. nigra* populations (e.g., [15,17,21,22]).

Plotting pairwise kinship coefficients for 15 distance classes under the even sample size option (Fig. 2) revealed no significant positive SGS in the studied population of *P. nigra*. Only one significant negative coefficient ($F_{ij} = -0.017$) was estimated in the distance class of 201–225 m. SGS in natural populations can result from different processes, including limited gene dispersal, selection pressure or historical events [11]. SGS analysis in the population of *P. nigra* near Dęblin indicated the lack of family structure. Both the seed and pollen of *P. nigra* are widely dispersed by wind, providing a simple explanation for the lack of SGS observed in our study. However, it is possible that the primary genetic structure of the population could be distorted by human activity.

The present study is the first report on the genetic diversity of *P. nigra* in Poland, and we believe the findings will be a source of inspiration for the creation of programs for the conservation and restoration of this species in our country.

Acknowledgments

The authors would like to thank for M. Ratajczak for providing expert technical support.

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