

Study on the introgression of hybrid poplar genes in the gene pool of black poplar in the Danube National Park



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Abstract

The black poplar (*Populus nigra*) is one of the characteristic species of the riparian forests. Plantation of hybrid poplars associated with the risk of hybrid poplar gene introgression into the gene pool of black poplar and the loss of habitat endanger the distribution of the black poplar.

The aim of this study is to analyze the genetic composition of the black poplar population located in the Danube National Park and to determine if hybrid poplar gene introgression in the black poplar gene pool takes place. The findings of this study are the basis for *in-situ* and *ex-situ* conservation of black poplars. In the course of this study, the genetic material of 317 offspring, 104 adult black poplars and 4 reference samples are investigated, paternity analysis is made and distribution-tendencies are illustrated.

The results show that introgression takes place and about 5-10 % of the offspring contain hybrid poplar genes.

Keywords

Black Poplar, Introgression, Danube National Park

Introduction

In 1998 / 1999, 1.532 sampling points were established throughout the area of the Danube National Park (Raster 400 m x 100 m). An angle count sampling accomplished in the Danube National Park during the winter months 2008 / 2009 determined 548 adult black poplars on 171 sampling points (ÖBF 2009; ECKMÜLLNER et al. 2008). On 24 of these 171 sampling points with black poplars, 101 hybrid poplars occurred (ÖBF 2009).

The research questions of this study are:

1. How many populations of black poplar can be determined in the Danube National Park?
2. Does introgression take place and if so, to what extent?
3. Is it possible to detect adult hybrid poplars as parents (parenthood analysis)?
4. Are there any distribution patterns?
5. What is the effect of a combination of different analysis methods with regard to the results?

Methods

Leaves of 105 adult poplars (dbh > 10 cm) and 318 offspring (dbh < 10 cm) were collected on 8 sample sites (Fig. 1) throughout the area of the Danube National Park. The adult poplars were located within a radius of about 100 m to the respective offspring. Adult hybrid poplars which could act as parent were also collected within this radius.

All individuals were marked and tagged with GPS. The DNA was extracted from the leaves with the Invisorb®Spin Plant Mini Kit.

439 samples (including reference samples) were analyzed by microsatellite markers to determine, if there is any influence of hybrid poplar genes.

Thus, 16 gene loci were examined: WIN3, wpms14, wpms17, wpms20, wpms09, wpms15, PMGC14, ORNL214, wpms16, PTR8, ORPM344, GCPM1894, ORPM29, ASP112322, GCPM1719 and PTR4.

The WIN3 analysis was done by agarose gel electrophoresis. For all further analysis methods, the fragment length of each gene loci was detected with the CEQ™ 8000 Genetic Analysis System (© Beckman Coulter).

All detected fragment lengths (exclusive the WIN3 gene locus) were noted in Excel. The values were compared to a reference list of fragment lengths provided by the Austrian Research Centre for Forests and compared to the fragment lengths of the reference samples. An individual was – mathematically – influenced by hybrid poplar genes when the occurred fragment lengths of one locus differed in both alleles (if existing) and one rare value occurred or when the fragment lengths of two gene loci were completely different from the reference list / reference samples.

The program R 3.3.2 (© The R Foundation) / RStudio 1.0.44 (© 2016 RStudio) Package PoppR 2.3.0 was used to put the individuals in various groups if more than 6 different fragment lengths on the analyzed gene loci / alleles between the compared individuals occurred. Individuals with 6 or less different fragment lengths were put together in one group.

Parenthood analysis was made with Cervus 3.0.7 (© Tristan Marshall 1998 – 2014 Field Genetic Ltd).

GenALEx 6.502 – Genetic Analysis in Excel (Peakall & Smouse 2006, 2012) was used to calculate the genetic distance of the samples, conduct a principal coordinate analysis and analyze the molecular variance within and between individuals.

The calculation of the possible amount of origin populations was effectuated by Structure 2.3.4. (PRITCHARD et al. 2000; FALUSH et al. 2003, 2007; HUBISZ et al. 2009). The data provided by Structure 2.3.4 were analyzed with Structure Harvester (DENT & VONHOLDT 2012) and Clumpak (KOPELMAN et al. 2015).

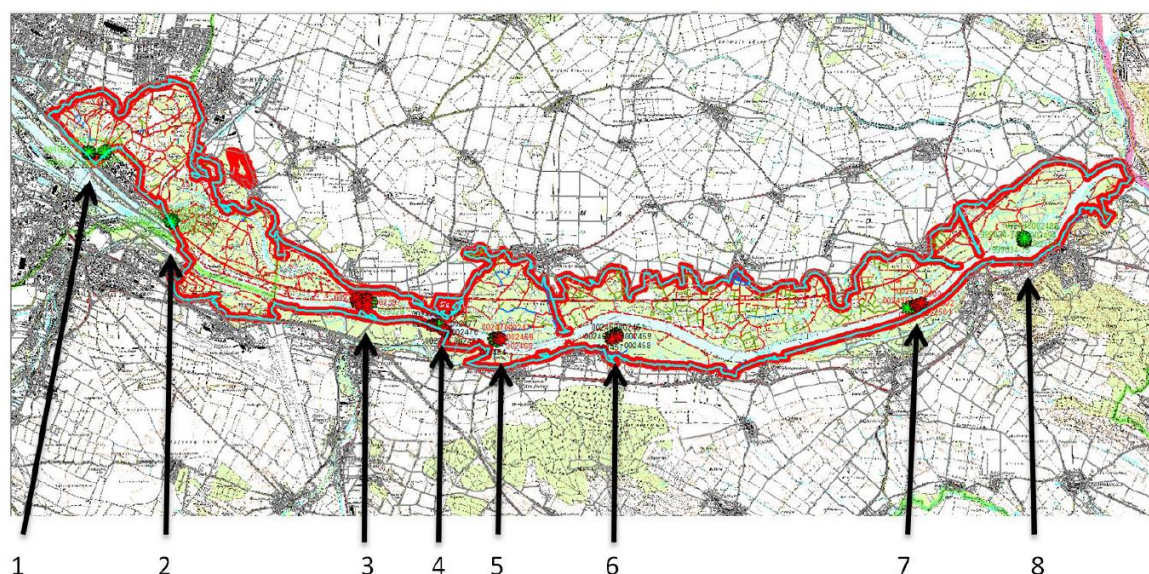


Figure 1: Location of the sampling sites. Red line = Border of the Danube National Park.

Results

1. Nowadays there is one single population of black poplar located in the Danube National Park. Gene flow takes place between the individuals throughout the whole area of the Danube National Park. Analysis show that this – nowadays single – population is composed of three origin populations.
2. 51 of the analyzed Individuals are influenced by hybrid poplar genes. From these 51 Individuals, 31 juveniles show an interference of hybrid poplar genes. Introgression takes place and about 9.8 % of the offspring is influenced by hybrid poplar genes.
3. 15 out of 22 sampled adult hybrid poplars were defined as parent-tree. 6.3 % of offspring could be assigned to a specific adult hybrid poplar.
4. 27.9 % of the offspring are located at the same sampling point as the designated parent-tree. 58.2 % of the investigated individuals show a difference of two sampling points between offspring / parent-tree.
5. Different methods of analysis showed a variable amount of individuals which were influenced by hybrid poplar genes. 29 of the individuals were found to be influenced in more than one analysis. 21 individuals were found to be influenced only by a single analysis method, independent of the method. Every analysis method shows - in some cases - different individuals which were determined as influenced by hybrid poplar genes.

Discussion

An allocation of one sample to a determined cluster in R does not mean that this sample is influenced by hybrid poplar genes. If samples are put in the same cluster it can be concluded that there is some kind of (family) relationship between the samples.

The combination of the WIN3 analysis and the allocation to a cluster in R give first clues if samples are influenced by hybrid poplar genes and if there is some kind of (family) relationship between these samples.

The results of the PCoA showed some kind of allocation of the samples in 3 different clusters with transition areas. It was not possible to detect a specific pattern within these clusters. It was not possible to find any correlation between age group and allocation to one cluster either. The molecular variance of 2 % between the sampling sites confirmed the suggestion that there is just one single population of black poplar which stands in genetic exchange throughout the whole area of the Danube National Park.

A mixture of genes between black and hybrid poplar across many generations could be excluded because of the analysis in Structure. The majority of analysed black poplar samples is not influenced by hybrid poplar genes.

Conclusion

The ascertained introgression accords to the results / presumptions of further studies (cf. HEINZE 2008). BENETKA et al. 1999 identified an introgression of about 9 % by spontaneous pollination in open nature.

It seems that the rate of introgression stabilizes by an amount from 7 – 10 %. To preserve the existing population of black poplar in the Danube National Park, it is necessary to renaturate the riparian forests and protect existing habitats. Specific removal of hybrid poplars is not necessary.

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