Gene flow between cultivated poplars and native black poplar (*Populus nigra* L.): a case study along the river Meuse on the Dutch–Belgian border

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Abstract

For the first time and in contrast with former studies, evidence is presented for introgression of genes of *Populus deltoides* in the offspring of open pollinated *P. nigra* females. Information regarding the genetic origin of 34 seeds produced by an isolated female of *P. nigra* and of 29 seedlings that spontaneously colonised the banks of the river Meuse along the Dutch–Belgian border, was obtained by using a combination of informative molecular markers. Thirty-two seedlings from the open pollinated *P. nigra* female were identified as hybrids.

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Keywords: *Populus*; Intra- and interspecific crossings; Introgression; Conservation

1. Introduction

European black poplar (*Populus nigra* L.) is a key species of the floodplain forest, which is the natural vegetation type on riverbanks in Western Europe. In common with many other European countries, floodplain areas in Belgium were subjected to urbanisation. Native poplar stands were displaced by agriculture or by cultivated poplar plantations consisting of a narrow range of euramerican (*P.* × *canadensis*) and interamerican (*P. interamericana* ¼ *P. deltoides* × *P. trichocarpa*) hybrids. There are concerns that massive introduction of genes of foreign species into the native *P. nigra* (i.e. introgression or introgressive hybridisation) could lower the effective population size and reduce the overall fitness of seedlings of the native *P. nigra* (e.g. Cagelli and Lefèvre, 1995).

It is not always possible to detect introgressed genes in the offspring of *P. nigra* on the basis of morphological traits alone (e.g. Heinze, 1997). Molecular markers based on isozymes, the codominant nuclear Sequence Tagged Site (STS) marker win3 (Bradshaw et al., 1994) and nuclear microsatellite markers (SSR)

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(Fossati et al., 2003) provide powerful new tools which can be used to assess the extent of (introgressive) hybridisation between introduced and wild relatives in open pollinated (OP) progenies of *P. nigra*. However, previous studies detected no evidence of introgression of *P. deltoides* into *P. nigra* by fertilisation of *P. nigra* females with pollen produced by trees of *P.* × *canadensis* growing in the vicinity (Heinze, 1997; Rajora, 1986; Janßen, 1998; Benetka et al., 1999; Tabbener and Cottrell, 2003; Fossati et al., 2003).

When used independently, these markers have the power to detect all F1 hybrids between *P. nigra* and *P. deltoides* but can fail to detect further generations of hybrids and backcrosses. However, the risks of inadequate sampling of the genome and of misinterpreting the data are greatly reduced when information from several molecular markers is combined (e.g. Heinze, 1997). In order to maximise their power to detect introgression we have therefore, combined the information from the following markers: isozymes, the STS marker (win3) and SSR markers.

The objectives of this study were to (i) determine if pollen gene flow occurred between male cultivated poplars (*P.* × *canadensis* and *P. nigra* var. *italica*) and female native black poplars, (ii) study the genetic origin of poplar seedlings that spontaneously colonised the river banks, and (iii) translate knowledge from this genetic study into practical guidelines for the restoration of black poplar along the Dutch–Belgian river Meuse.

2. Material and methods

2.1. Survey and collection of plant material

The riverside along 45 km of the Meuse on the Dutch–Belgian border from Lanaken to Kessenich on the Belgium side and from Maastricht to Roosteren, on the Dutch side was surveyed in order to locate the remaining adult, reproductive relict individuals of black poplar in the area. Winter cuttings were collected from the relict individuals and grown in the greenhouse to produce young leaf material for DNA-extraction and isozyme analysis. Thirty-four OP seeds were collected during spring 1999, from a veteran black poplar tree located in Bilzen ($50^{0}51^{00}26^{00}$N/ $05^{0}30^{0}40^{00}$E). It was surrounded by flowering plantations of *P.* × *canadensis* hybrids within a distance of 5 m. Three flowering individuals of the male *P. nigra* var. *italica* were located at a distance of 50 m and the nearest known indigenous male black poplar grew 25 km away. Seeds were sown in trays in the greenhouse within 24 h of collection. In 2001, 33 seedlings that had spontaneously colonised the gravel banks were dug out and potted up in the greenhouse and young leaves were collected from the 29 seedlings, which survived. These were used immediately for isozyme analysis, and lyophilised prior to DNA-extraction.

2.2. Molecular methods and data analysis

Analysis of isozyme systems PGI, PGM and LAP which are diagnostic for discrimination between *P. deltoides*, *P. nigra* and their hybrids (Rajora, 1989), was performed as described by Hochu and Fady (1998). Following extraction with the Dneasy Plant Miniprep Kit (Qiagen, Helden, Germany) genomic DNA was analysed using the species-specific co-dominant STS marker (win3) (Heinze, 1997). All samples and *P. nigra* var. *italica* were analysed using the following five nuclear microsatellite loci: PMGC14 (http://poplar2.cfr.washington.edu/pmgc), WPMS09, WPMS16, WPMS14, WPMS20 (Van der Schoot et al., 2000; Smulders et al., 2001). For SSR-analyses, fragment separation was performed on an ABI 310 Prism Genetic Analyser (Perkin Elmer—Applied Biosystems). The software programs Genescan and Genotyper 2.5 (PE-Applied Biosystems, Foster City, CA) were used to process and score the SSR data. A model-based clustering method was applied to SSR data to infer genetic structure and define the number of genetic clusters (*K*) in the dataset using the software STRUCTURE (Pritchard et al., 2000). The following reference samples were analysed to generate reference groups: *P. nigra* var. *Wolterson*, *Neuburg*, *Vereecken* and *Tilff*; *P. deltoides* var. *Harvard*, *Peoria* and *S.9-2; P. trichocarpa* var. *V23*, *V24*, *V235*, *Blom* and *Trichobel*; *P.* × *canadensis* var. *Gaver*, *Ghoy*, *Gelrica*, *Robusta*, *Gibecq*, *Isière*, *Ogy* and *Primo* and *P. deltoides* × *P. trichocarpa* var. *Beaupré*, *Boelare*, *Hazendans*, *Hoogvorst* and *Unal*. SSR data were also used to determine, by exclusion analysis, whether *P. nigra* var. *italica* could be the

father of any of the progeny (Vanden Broeck et al., 2002).

3. Results and discussion

Our survey discovered seven adult black poplar trees at three locations within 1 km of the banks of the river Meuse. These consisted of the following; a single female near Lanaken, two females at Meers and four males at Geleen. Further away, at a distance of 15 km from the river Meuse at Bilzen there were an additional two females. As the sex and the multilocus SSR genotypes of trees at a single location were identical it was concluded that there was only a single genotype at each location (Storme et al., 2002). In contrast to native black poplar, plantations of exotic poplars of *P.* × *canadensis* and *P. nigra* var. *italica* were very common; it was possible to see exotic poplars from every position within the study area.

By combining the data of five co-dominant diagnostic markers (three isozyme markers, microsatellite WPMS09 and win3) we could detect a species-specific allele of *P. deltoides* in 32/34 seeds of the OP progeny. These 32 seeds were heterozygous for at least 1 locus showing 1 allele specific for *P. deltoides* and another specific for *P. nigra*. Based on the separate data of the diagnostic markers WPMS09 and win3, introgression of *P. deltoides* and heterozygosity was detected in 17/34 and 16/34 individuals of the OP progeny, respectively. Despite the rather small sample size, our results clearly show that spontaneous hybridisation occurs between female *P. nigra* and male *P.* × *canadensis*. This contrasts with former studies in which *P.* × *canadensis* growing in the vicinity failed to act as father to any of the offspring of *P. nigra* females that were tested. The fact that hybridisation in controlled conditions between *P. deltoides* and *P. nigra* is only possible when *P. deltoides* is the female parent (Zsuffa, 1974) is often discussed as a possible reason for the lack of introgression of genes of *P. deltoides* in OP progenies of *P. nigra* (Rajora, 1986; Benetka et al., 1999). However, our results show that genetic incompatibility does not prevent natural hybridisation between male *P.* × *canadensis* and female *P. nigra*. In our study, the nearest male *P. nigra* was 25 km away from the female and therefore there is likely to be very low levels of *P. nigra* pollen available for the *P. nigra* female. This is in contrast with former studies where indigenous male black poplars grew close to the female trees. It would therefore appear that pollen of *P.* × *canadensis* is only successful when there is very little *P. nigra* present in the pollen cloud (Vanden Broeck et al., 2002; Tabbener and Cottrell, 2003).

Table 1
Summary of the results obtained using the assignment procedure based on SSR data of all samples (including references)

Category of origin	*N*	Inferred gene pools			
		1	2	3	4
P. deltoides	3	0.011	0.035	0.937	0.017
P. trichocarpa	5	0.006	0.156	0.007	0.830
P. nigra (references þ relict trees)	13	0.461	0.450	0.084	0.005
P. × *canadensis*	8	0.044	0.622	0.284	0.050
P. trichocarpa × *P. deltoides*	5	0.009	0.046	0.0018	0.928
Progeny 1999	34	0.911	0.047	0.028	0.014
Seedlings gravel banks	29	0.072	0.258	0.474	0.197

Figures are the proportion of estimated membership to each of the four inferred gene pools for genotypes of a given category of origin (*N* ¼ number of samples).

When the model-based clustering method of Pritchard et al. (2000) was applied to the SSR data, the highest estimate of the likelihood of the data, conditional on a given number of clusters, was obtained when all genotypes were clustered into four gene pools.

The results of the assignment test (Table 1) indicate that at least a part of the genetic information from the seedlings from the gravel banks originates from non-native poplar species. This indicate that cultivated poplars are reproductive in the study area. Some of the introgressed seedlings seemed to be well adapted as they had survived the river dynamics over several years. They may compete with the native species in colonising new habitats.

Using SSR markers, it was possible to exclude *P. nigra* var. *italica* as a potential male parent in 62 of the 63 seeds and seedlings that were tested. The sample that may have been fathered by *P. nigra* var. *italica* was a seedling collected on the gravel banks. Further research is needed to study introgression by *P. nigra* var. *italica*. Apparently, the widely planted cultivar, does not pose a severe threat to the integrity of the Belgian *P. nigra* gene pool, probably due to its early flower phenology.

Our results have practical implications for *in situ* conservation and for the restoration of natural

populations of black poplar. Low levels of introgression are expected in natural populations of black poplar where male black poplars grow in close proximity to female trees. However, caution should be exercised when isolated female black poplar trees are surrounded by interspecific hybrid males, which may be a source for introgression in the native populations. This study also provides insights in the potential for transgene flow of genetically engineered poplar plantations as this might depend heavily on whether conspecific pollen of the native species is available.

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