Genetic traces of cultivated hybrid poplars in the offspring of native *Populus nigra* in Austria

Genetické stopy hybridních kultivarů v potomstvu původního druhu *Populus nigra*

Berthold Heinze

Department of Genetics, Austrian Federal Research Centre for Forests, Hauptstrasse 7, A-1140 Vienna, Austria, e-mail: Berthold.Heinze@bfw.gv.at

Heinze B. (2008): Genetic traces of cultivated hybrid poplars in the offspring of native *Populus nigra* in Austria. – Preslia 80: 365–374.

Hybrid poplars like *Populus* ×*canadensis* or balsam poplar (*Populus* section *Tacamahaca*) hybrids have been propagated in Europe since the early 18th century. They replaced many stands of native black poplar on the banks of the major rivers. While spontaneous crosses between hybrid and native black poplars were not considered to be common or of importance in nature, it is shown that such crosses have occurred in Austria, as *P. deltoides* and section *Tacamahaca* alleles (PCR-amplified chloroplast and nuclear DNA markers) were found in plants morphologically similar to *P. nigra*. In the localities studied, a realistic estimate of the introgression rate is between 0 and 10 % of plants in a given stand. Female hybrid trees produce viable seed. This shows that hybrid poplars can spread their genes by sexual means, which may result in continuing introgression and consequently, a reduction in genetic diversity and fitness of the endangered *P. nigra*.

Keywords: chloroplast markers, genetic conservation, introgression, PCR-RFLP, *Populus* hybrids, *Populus nigra*

Introduction

Floodplain forests in Europe once covered vast areas in the lowlands and river valleys and supported a high level of biodiversity. These forests are now fragmented and greatly reduced in size due to human activity. In some countries like the Netherlands or Great Britain, they have disappeared completely. Black poplar (*Populus nigra*) is a keystone species in this habitat: as a pioneer tree, it quickly covers bare soil exposed by flooding, grows to considerable size and forms dense stands that consolidate and change the site in favour of longer-lived trees like elms and oaks (Lefèvre et al. 2001b).

The management of the remaining floodplain forests in Europe, or their restoration, is made more difficult by the large, planted, monoclonal hybrid poplar stands. Evenly spaced rows of similar looking hybrid poplar trees are considered unnatural by the general public, but the real threat they pose to conservation is probably more subtle. Hybrid poplars are intermediate in many morphological features between *P. nigra* and their other respective parents. For instance, the widely planted *P.* ×*canadensis* hybrids are offspring of crosses between female *P. deltoides* and male *P. nigra* (Zsuffa 1974, Heinze 1998b). Crosses among hybrid clones, or backcrosses to *P. nigra*, may be difficult to identify among "pure" black poplar offspring, as traits are highly variable within this species (EUFORGEN 2007). Poplars are dioecious, each clone only produces either pollen or seed. A limited number of hybrid poplar clones are currently planted (Pinon & Valadon 1997). The mass cultivation of hybrid poplar clones, together with the diminishing number and fragmentation of black poplar populations, may lead to introgression and consequently a severe reduction in genetic variation of this native species (Lefèvre et al. 2001a,b). The purpose of this study was to assess whether introgression occurred in poplars sampled throughout the species range in Austria, by screening for molecular markers specific to poplar hybrids.

Materials and methods

Plant material from naturally established trees was collected (winter buds and leaves; seed was collected from the ground or harvested from single trees and germinated) at a number of locations (Table 1) in or close to the following river valleys in Austria: Salzach/Inn (18 trees), Danube (351 trees, 174 seeds), Traisen (7 trees, 3 seedlings), Kamp (6 trees), Mur (40 trees) and Kainach (1 tree). All but the Mur and Kainach rivers join the Danube river in Austria north of the Alps; the latter two rivers (the Kainach joins the Mur near Graz) leave the Alps in a southerly direction, but also eventually join the Danube. Additionally, seed was collected from hybrids: a solitary hybrid poplar tree of unknown parentage (Mariabrunn arboretum, Wien river, which joins the Danube in Vienna, 47 seedlings) and a *P.* ×*canadensis* hybrid poplar plantation (Melk, 23 seedlings). Sampling in the field was mainly during winter, as dormant buds are the best source of DNA. However, for this reason it was not possible to make a full morphological characterization, especially of the leaves. Morphology was checked with the help of the EUFORGEN *P. nigra* identification sheet (EUFORGEN 2007).

Chloroplast PCR-RFLP markers (Darling & Blum 2007), which are maternally inherited in *Populus*, as confirmed by Mejnartowicz (1991), as well as nuclear DNA markers of bi-parental inheritance, were analysed. DNA extractions and PCR conditions were essentially as previously described (Heinze 1997, Heinze 1998a, b). Chloroplast DNA markers were obtained using "universal" chloroplast primers (Heinze 2007) for the genes *trn*T-*trn*D, *trn*S-*trn*fM (Demesure et al. 1995) and *trn*G-*ycf*9 (= ORF62; Heinze 1998a), and digesting amplification products using restriction enzymes *Eco*RI, *Hinf*I and *Rsa*I (details as in Heinze 1998b). Nuclear DNA markers were developed from the poplar DNA sequences reported by Bradshaw et al. (1994; win3, see Heinze 1997) or present in Genbank: primers PPAL-P1 and –M1 based on phenyl alanine-ammonia lyase (GenBank accession no. POPPALGA; primer nucleotide sequences 5'-3', CAA GGC GGT GCT CTT CAG AAG G and GCT GAG TGA GGC AGT GTG TGG C, respectively; annealing temperature 65 °C) and POPX-P5 and POPX-M25 based on aniodic peroxidase (accession no. POPP02; primer sequences, GTC CAA ATG TGA GCA GCA TTA TCC and ATC AGT CTG AAG CAG GCC TCG, respectively; annealing temperature 62 °C) were designed. The DNA fragment produced by the PPAL primers was digested using restriction enzyme *Hae* III, and the POPX fragment using *Msp* I, which reveals species-specific bands after standard agarose electrophoresis. Alleles revealed by these markers can be assigned to either *P. nigra*, *P. deltoides*, or species of section *Tacamahaca* (*P. trichocarpa*, *P. maximowiczii*) unambiguously. Using these markers, introgression and the maternal lineage of introgressed plants can be determined (Heinze 1998b).

Table 1. – Plant material analysed for introgression. Seeds were picked from single trees, or collected from the ground under groups of trees. Seedlings were raised from seeds in the BFW nursery.

River system (total number of samples)	Location	Number of plants analysed	Type of plant	Material collected
Salzach/Inn (18)	Anthering	7	adult trees	leaves
	Ostermiething – Ettenau	3	adult trees	leaves
	Mühlheim	8	adult trees	leaves
Danube (595)	Feldkirchen	2	adult trees	winter buds
	Traun	17	adult trees	winter buds
	Puchenau	1	adult tree	winter buds
	Steyr	3	adult trees	winter buds
	Grafenegg Seebarn	50	adult trees	winter buds
	Klosterneuburg	16	adult trees	winter buds
	Schwarzlackenau	36	young trees	winter buds
	Brigittaspitz	10	seeds of adult trees	leaves of seedlings
	Donaukanal in Wien-Heiligenstadt, near Spittelau station	17	seeds of single young tree	leaves of seedlings
	Donaukanal in Wien-Heiligenstadt, near Spittelau station	8	young trees	winter buds
	Donauinsel	31	seeds of young trees	leaves of seedlings
	Mariabrunn Wienflußaufsicht	45	seeds of single adult tree	winter buds
	Das Schwarze Loch Lobau	12	seeds of adult trees	leaves
	Lobau, *P. nigra* near hybrid poplar plantation	16	seeds of adult trees	leaves of seedlings
	Lettenhaufen	53	adult trees	winter buds
	Untere Lobau	19	adult trees	winter buds
	Lobau Kreuzgrund	18	seeds of adult trees	leaves of seedlings
	Lobau general	10	adult trees	winter buds
	Albern	39	adult trees	winter buds
	Mannswörth	50	young trees	winter buds
	Fischamend	1	adult trees	winter buds
	Haslau	8	adult trees	winter buds
	Petronell	15	adult trees	winter buds
	Hainburg	10	adult trees	winter buds
	Eckartsau	25	seedlings	winter buds of seedlings in local nursery
	Eckartsau	13	adult trees	winter buds
Traisen (10)	Ochsenburg	3	seeds of adult trees	leaves of seedlings
	Preuwitz	7	adult trees	winter buds
Kamp (6)	Zöbing	2	adult trees	winter buds
	Stiefern, Schönberg	2	adult trees	winter buds
	Krems	2	adult trees	winter buds
Mur (40)	between Leoben and Bruck	40	adult trees	winter buds
Kainach (1)	St. Johann – Flecker-Kreuz	1	adult tree	winter buds
Wien (seeds of hybrid; 47)	Mariabrunn	47	seed collected from solitary hybrid tree	leaves of seedlings
Danube (seeds of hybrids; 23)	Melk	23	seeds collected in hybrid plantation	leaves of seedlings

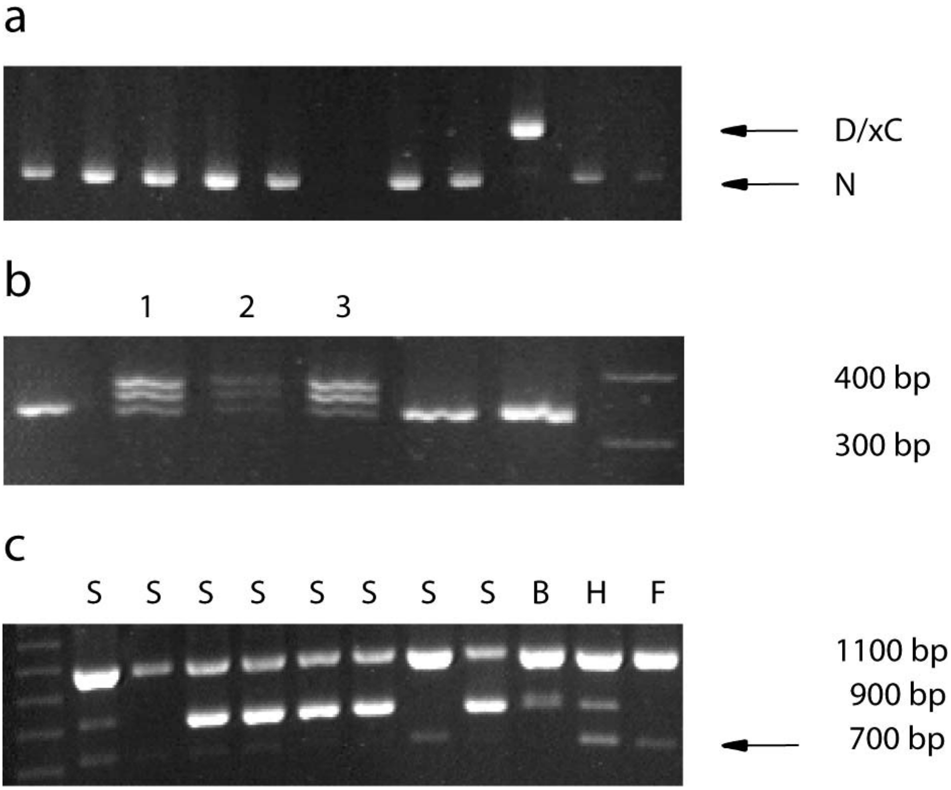

Fig. 1. – Hybrid poplar genes introgressed into black poplar plants: (a) tree Klosterneuburg 14 with *Populus deltoides / P.* ×*canadensis* chloroplast DNA type (D/xC, approx. 800 basepairs (bp)), among *P. nigra* trees (N, approx. 1000 bp). (b) Three trees (Grafenegg 4–6, lanes 1–3) with one *P. nigra* and presumably one *P. deltoides* PPAL/Hae III allele (and one heteroduplex DNA band, see Heinze 1997), among true-breeding *P. nigra*. (c) Balsam poplar POPX/MspI alleles (arrow, approx. 700 bp) introgressed in a female (F) and some seedlings (S), at Heiligenstadt. H, a *P. nigra* × *P. maximowiczii* hybrid growing close by, is the possible source of these alleles. B, *P. laurifolia* × *P. nigra* hybrid 'Berolinensis', also present at this site in large numbers, is a less likely pollen donor.

Results

In total, 525 trees or seedlings, which were expected to be more or less pure *P. nigra*, and 38 seedlings from *P.* ×*canadensis* hybrid trees were analysed. While most of the 525 “natural” plants showed marker constellations typical of *P. nigra* (504 plants), a number of trees or seedlings harboured *P. deltoides* or balsam poplar alleles (Table 2). There were three categories of deviant plants: (i) typical F1 hybrid poplars (e.g., Klosterneuburg 14, Albern 10, 13, 41, Donaukanal in Wien-Heiligenstadt tree 1), (ii) plants raised from seeds collected under or in the vicinity of hybrid poplars (e.g., Melk, Mariabrunn, Donaukanal in Wien-Heiligenstadt seedlings), which showed segregation of the alleles of the two parent species and (iii) single trees with some alleles from *P. deltoides* and some from *P. nigra* in no fixed pattern. In the first case, these are presumably true F1 hybrid poplars that were planted, or developed from suckers from rootstocks of felled trees. Genetic fingerprinting (e.g., Castiglione et al. 1993, Fossati et al. 2003) may reveal the clonal identity of these plants. Only one of the typical F1 hybrids at Donaukanal in Wien-Heiligenstadt, a relatively young female tree (estimated age around 15 years) growing very close to the Danube canal in Vienna, had apparently grown spontaneously from seed. In the second case, plants from seed collected from hybrids had combinations of nuclear alleles from the two parent species, together with chloroplast DNA of either *P. deltoides* (Melk, Mariabrunn), *P. nigra*, or balsam poplar (seedlings from Donaukanal in Wien-Heiligenstadt), as expected if there had been crossings among F1 hybrids and backcrosses with *P. nigra*. The third case is the most interesting: five plants in two naturally established stands were identified as backcrosses of hybrids with *P. nigra* (Table 2). While Mannswörth 45 and 48, young trees in a population on a former building site, are from seed of a hybrid poplar mother, the three deviant trees at Grafenegg possibly developed from seed produced by female *P. nigra* fertilized by a male hybrid poplar. An alternative explanation for their banding patterns may be an as yet undetected alternative *P. nigra* allele at this locus. These plants were found among black poplar trees, which indicated that backcross plants can survive and compete successfully with genetically "pure" individuals.

Table 2. – Genes of hybrid poplars detected by genetic analysis. Given for each population is its location, designation and description.

Population	Genetic analysis
Grafenegg/ Seebarn 4, 5, 6: three trees in mature stand (50 trees sampled)	*P. nigra* chloroplast and *P. ×canadensis* PPAL alleles
Klosterneuburg 14: one mature tree in native black poplar stand (16 trees sampled)	*P. deltoides* chloroplast, *P. ×canadensis* nuclear allele combinations
Donaukanal in Wien-Heiligenstadt tree 1: young female tree on the edge of Danube canal	*P. nigra* chloroplast, *P. nigra*/balsam poplar nuclear allele combination (marker POPX/MspI)
Donaukanal in Wien-Heiligenstadt seedlings 1–8: seeds collected near tree Donaukanal in Wien-Heiligenstadt 1	*P. nigra* and balsam poplar chloroplasts, nuclear alleles pure *P. nigra* or *P. nigra*/balsam polpar combination (markers POPX/MspI, PPAL/RsaI)
Albern 10, 13, 41: three mature trees in native black poplar stand (all 39 trees in the stand were sampled)	*P. deltoides* chloroplast, *P. ×canadensis* nuclear allele combinations
Mannswörth 45, 48: two young trees among black poplars at a regeneration site (50 trees sampled)	*P. deltoides* chloroplast, *P. nigra* and *P. ×canadensis* nuclear allele combinations
Mur, Pux-Au 4, 6, 7: three trees of a stand outside the natural distribution range	*P. deltoides* chloroplast, *P. ×canadensis* nuclear allele combinations
Mariabrunn hybrid poplar Südgarten 1–15: seeds from solitary hybrid tree	all have *P. deltoides* chloroplast with *P. nigra, P. ×canadensis* or *P. deltoides* nuclear allele combinations
Melk 1–24: seeds from hybrid plantation	all have *P. deltoides* chloroplast *with P. nigra, P. ×canadensis* or *P. deltoides* nuclear allele combinations

Discussion

The results clearly demonstrate that hybrid poplars, previously thought to be dependent on man for vegetative propagation, can (i) compete, by suckering, with naturally established seedlings, and (ii) also propagate sexually and introgress with the native European species, *P. nigra.* The identification of such individuals based on morphological traits is difficult, especially saplings in winter (EUFORGEN 2007). The majority of the plants investigated were "true" black poplars, which accords with the morphological assessment. On the basis of this investigation (Table 2), estimates of introgression percentages ranged between zero and ten in the areas studied in Austria. These estimates include an allowance for some undetected hybrid alleles, either due to incomplete sampling of the stands, or incomplete genomic coverage.

The methods used in this study were suitable for very small seedlings. Poplar seed is short-lived, and seedlings may quickly disappear if the site dries out. Using DNA markers, these seedlings can be studied long before they develop morphologically distinctive features, which usually takes at least a number of years. Not even isoenzyme (allozyme) analysis can be done with as little as a single poplar cotyledon.

The occurrence of introgression in these species was suspected on the basis of results of chloroplast DNA studies in Germany (Vornam et al. 1994), isoenzyme studies in France (Legionnet & Lefèvre 1996), and AFLP marker results from the Netherlands (Arens et al. 1998); it was also known to occur in other closely related *Populus* species in North America (Eckenwalder 1982, Meirmans et al. 2007). A study of introgressed genes in German poplar populations using isoenzymes (Janßen 1998) obtained similar estimates to those reported in this study. The combination of chloroplast and nuclear DNA data used in this study provided evidence that hybrid and black poplars do indeed interbreed in both directions, which does not accord with that reported previously (e.g., Benetka et al. 1999, Holderegger et al. 2005), but does confirm with two recent reports from more northern locations in Europe (Vanden Broeck et al. 2004, Ziegenhagen et al. 2008). It seems that hybrids are more successfully propagated by pollinating hybrid females with *P. nigra* pollen (Benetka et al. 2002), but occasionally, male hybrids, when locally abundant, as at the Donaukanal in Wien-Heiligenstadt, can also successfully pollinate *P. nigra* females. The idea that solitary females of *P. nigra* are more prone to pollination by male hybrids is reasonable and now has experimental support from pollination trials (Vanden Broeck et al. 2003b). Benetka et al. (2002) have shown that *P. nigra* females produce approximately 7% introgressed offspring when fertilized with mixed pollen (*P. nigra* and *P. ×canadensis*). This is close to the estimates obtained in this study at some field locations.

There was no correlation between introgression and age of the trees; plants influenced by hybridization were present in all age classes. The contribution of hybrid and black poplars to pollination, and their respective densities, could not be assessed in the field. Poplar pollen and seed is blown over considerable distances by the wind, and possible progenitor populations of the older populations sampled possibly no longer exist. In general, however, crosses among black poplars may be advantageous, as monoclonal blocks of hybrid poplars with dense crowns often show reduced flower production, and presumably flower over a shorter period compared to multi-genotype native black poplar stands (Heinze & Lickl 2002). There may also be a qualitative (but not absolute) preference for pollen from the "pure" species (Vanden Broeck et al. 2003b, Benetka et al. 2002).

An estimate of propagule pressure is difficult to establish. Hybrid poplar plantations cover much more ground than native poplar stands in Central Europe. Nevertheless, at most of the locations studied, native black poplar seedlings were much more common than introgressed individuals. Sites with potential black poplar regeneration were specifically targeted in this study; a different picture will emerge where native black poplars are completely absent. Whether seedlings of native poplar enjoy a fitness advantage over introgressed individuals is equally difficult to judge. Poplar breeders have often argued that crosses between the two species beyond the F1 generation perform poorly (Zsuffa 1974). This was linked to hybrid heterosis in the F1, and its breakdown in F2 or backcrosses, most probably due to segregation distortion and genetic load (Bradshaw & Stettler 1994). However, recently released hybrid clones have more complex breeding histories, including backcrosses and crosses among different hybrids (S. M. G. de Vries, J. Van Slycken & L. Vietto, personal communications). The effect of hybrid alleles on invasiveness is even more difficult to assess. Wolfe et al. (2007) have found no such effect in a study of intraspecific F1 hybrids of the short-lived perennial plant, *Silene latifolia*. However, the situation in poplar is genetically more complex, and allele combinations favouring increased invasiveness may segregate in later generations, if high numbers of allele combinations (propagules) are released. Poplars are able to produce extremely high numbers of seeds and seedling establishment is a limiting factor. If in the future, larger areas are managed for poplar regeneration (Lefèvre et al. 2001a), we might expect some poplars to have more invasive characteristics as a consequence.

In the Danube valley around Vienna, mass cultivation of hybrid poplars started around 1860 (Jelem 1974). In spite of the relatively short time that has elapsed since then, in terms of tree generations, there is a considerable percentage of introgressed trees in some populations. Without clear and decisive counter-measures, this phenomenon will increase in momentum. Suitable sites for poplar regeneration are very rare, and hybrids are present nearly everywhere. The newly established national park on the Danube floodplains downstream of Vienna, for instance, is home to vast hybrid poplar plantings. Management regulations for this area must take into consideration the impact of sexual propagation of the hybrids (Lefèvre et al. 2001a); non-sterile female cultivars should be the the first to be removed, followed by other hybrids, especially those planted in the vicinity of regenerating native *P. nigra*.

A comparison with similar situations in other parts of Europe: Belgium (Vanden Broeck 2004), N Italy (Fossati et al. 2003), the Czech Republic (Benetka et al. 1999, 2002), N England (Gilbert 1993) and central Germany (Ziegenhagen et al. 2008) highlights the apparent dependence of introgression levels on local factors such as flowering phenology (Vanden Broeck 2003a, Heinze & Lickl 2002), the number and sex of remaining black poplars, and the hybrid clones present locally (Fossati et al. 2003, Ziegenhagen et al. 2008, Benetka et al. 1999, Gilbert 1993). On the northern edge of the distribution range of black poplar, hybrid poplars flower too early to cross-pollinate significantly (Vanden Broeck 2003a), but it remains to be seen whether this holds every year and in changing meteorological conditions. Lack of conspecific pollination partners (solitary female situation) seems to result in significant amounts of backcross offspring (this study; Vanden Broeck et al. 2004, Ziegenhagen et al. 2008). Offspring of female hybrids have been found several times in Europe (e.g., this study, Ziegenhagen et al. 2008) and it would be interesting to investigate whether introgressed offspring can compete with native poplar seedlings, as this cannot be determined from the available data.

The recent publication on the genome of a poplar species (Tuskan et al. 2006) will enable the study of the genetic consequences of introgression (e.g., Meirmans et al. 2007, Fossati et al. 2004, Lexer et al. 2005, 2007, Van Loo et al. 2008 for the *P. alba*/*P. tremula* hybrid system). Microsatellites are already employed in introgression studies (e.g., Fossati et al. 2003, Vanden Broeck et al. 2004). They are on the one hand an example of what Darling & Blum (2007) call a "technological overkill for a given application" – for example, Khasa et al. (2005) screened 71 microsatellite loci in order to distinguish between native and introduced poplars in E North America, of which only five had non-overlapping size ranges. On the other hand, they may be ideal markers for establishing the exact paternity of introgressed individuals. For example, the contribution of the omnipresent Lombardy poplar *P. nigra* 'Italica', a male ornamental, to *P. nigra* offspring could be studied with their help. It may also be possible to track the most prolific hybrid cultivars and mark them for special attention.

In the future, it may be a desirable breeding objective to produce clones of poplars that are sterile, either by introducing triploidy or even by the use of genetic engineering. The latter may even be supported by nature conservationists. The first genetically modified hybrid poplars will presumably be planted on a large scale in the near future. The genetically modified female clones will increase the risk of spreading transgenes, which could be reduced if male clones are planted.

In summary, this study confirms that offspring of planted poplar hybrids are able to invade the last remaining sites where the native black poplar regenerates, and that the genome of the native species is being invaded by alien genes. The extent of the introgression seems to depend very much on local conditions, calling for a careful case-by-case assessment.

Acknowledgements

I thank all landowners for access and permission to sample plants, and E. Kickingereder for laboratory work. Tony Dixon kindly improved my English.

Souhrn

Hybridní topoly jako *Populus* ×*canadensis* nebo taxony ze sekce *Tacamahaca* se v Evropě pěstují a rozšiřují od začátku 18. století. Na mnoha březích velkých řek nahradily porosty původních druhů topolů. Dosud se předpokládalo, že ke spontánní hybridizaci mezi původními druhy a těmito hybridy nedochází příliš často, a nebyla jí tudíž věnována pozornost; tato studie však ukazuje, že v Rakousku probíhá. U rostlin morfologicky podobných *P. nigra* byly zjišteny alely *P. deltoides* a taxonů ze sekce *Tacamahaca* (chloroplastové a jaderné DNA markery, amplifikované metodou PCR). Realistický odhad míry introgrese na studovaných lokalitách se pohybuje mezi 0–10%. Samičí hybridní stromy vytvářejí životaschopná semena. Hybridní topoly mohou šířit svoje geny pohlavním rozmnožováním, což může vést k pokračující introgresi a snížit genetickou diverzitu a zdatnost ohroženého druhu *P. nigra*.

References

Arens P., Coops H., Jansen J. & Vosman B. (1998): Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. – Mol. Ecol. 7: 11–18.

Benetka V., Mottl J., Vacková K., Pospíšková M. & Dubský M. (1999): Estimation of the introgression level in *Populus nigra* L. populations by means of isozyme gene markers. – Silvae Genetica 48: 218–223.

Benetka V., Vacková K., Bartáková I., Pospíšková M. & Rasl M. (2002): Introgression in black poplar (*Populus nigra* L. ssp. *nigra*) and its transmission. – J. Forest Sci. (Prague) 48: 115–120.

Bradshaw H. D. jr. & Stettler R. F. (1994): Molecular genetics of growth and development in *Populus*. II. Segregation distortion due to genetic load. – Theor. Appl. Genet. 89: 551–558.

Bradshaw H. D. jr., Villar M., Watson B. D., Otto K. G., Stewart S. & Stettler R. F. (1994): Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. – Theor. Appl. Genet. 89: 167–178.

Castiglione S., Wang G., Damiani G., Bandi C., Bisoffi S. & Sala F. (1993): RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp) clones. – Theor. Appl. Genet. 87: 54–59.

Darling J. A. & Blum M. J. (2007): DNA-based methods for monitoring invasive species: a review and prospectus. – Biol. Inv. 9: 751–765.

Demesure B., Sodzi N. & Petit R. J. (1995): A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. – Mol. Ecol. 4: 129–131.

Eckenwalder J. E. (1982): *Populus* × *inopina* hybr. nov. (*Salicaceae*), a natural hybrid between the native North American *P. fremontii* S. Watson and the introduced Eurasian *P. nigra* L. – Madroño 29: 67–78.

EUFORGEN (2007): *Populus nigra* identification sheet. Bioversity International, Rome, Italy. – URL: [http://www.bioversityinternational.org/Networks/Euforgen/Networks/Scattered_Broadleaves/PNOuputs/LEAFLE-E.PDF, accessed 10 August 2007].

Fossati T., Grassi F., Sala F. & Castiglione S. (2003): Molecular analysis of natural populations of *Populus nigra* L. intermingled with cultivated hybrids. – Mol. Ecol. 12: 2033–2043.

Fossati T., Patrignani G., Zapelli I., Sabatti M., Sala F., & Castiglione S. (2004): Development of molecular markers to assess the level of introgression of *Populus tremula* into *P. alba* natural populations. – Plant Breeding 123: 382–385.

Gilbert O. L. (1993): Regenerating balsam poplar (*Populus candicans* Ait.) x black poplar (*P. nigra* L.) (Salicaceae) at a site in Leeds. – Watsonia 19: 188–190.

Heinze B. (1997): A PCR marker for a *Populus deltoides* allele and its use in studying introgression with native European *Populus nigra*. – Belgian J. Bot. 129: 123–130.

Heinze B. (1998a): PCR-based chloroplast DNA assays for the identification of native *Populus nigra* and introduced poplar hybrids in Europe. – Forest Genet. 5: 31–38.

Heinze B. (1998b): Molekulargenetische Unterscheidung und Identifizierung von Schwarzpappeln und Hybridpappelklonen. – FBVA-Berichte 105, Federal Forest Research Centre, Vienna, Austria.

Heinze B. (2007): A database of PCR primers for the chloroplast genomes of higher plants. – Plant Methods 3: 4.

Heinze B. & Lickl E. (2002): Rare, but steady, introgression in Austrian black poplar as a long-term risk? – In: van Dam B. & Bordács S. (eds), Genetic diversity in river populations of European black poplar: implications for riparian eco-system management, Proc. Intern. Symp. held in Szekszárd, Hungary, 16–20 May, 2001, p. 169–175, Csiszár Nyomda, Budapest.

Holderegger R., Angelone S., Brodbeck S., Csencsics D., Gugerli F., Hoebee S. & Finkeldey R. (2005): Application of genetic markers to the discrimination of European Black Poplar (*Populus nigra*) from American Black Poplar (*P. deltoides*) and Hybrid Poplars (*P. x canadensis*) in Switzerland. – Trees – Structure and Function 19: 743–748.

Janßen A. (1998): Artbestimmung von Schwarzpappeln (*Populus nigra* L.) mit Hilfe von Isoenzymmustern und Überprüfung der Methode an Altbäumen, Absaaten von kontrollierten Kreuzungen und freien Abblüten sowie Naturverjüngungen. – Forschungsberichte der Hessischen Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie 24, p. 32–42, Hann. Münden, Germany.

Jelem H. (1974): Die Auwälder der Donau in Österreich. – Mitt. Forstl. Bundes-Versuchsanst. Mariabrunn 109: 1–287.

Khasa D., Pollefeys P., Navarro-Quezada A., Perinet P. & Bousquet J. (2005): Species-specific microsatellite markers to monitor gene flow between exotic poplars and their natural relatives in eastern North America. – Mol. Ecol. Notes 5: 920–923.

Lefèvre F., Barsoum N., Heinze B., Kajba D., Rotach P., de Vries S. M. G. & Turok J. (2001a): In Situ Conservation of *Populus nigra*. – EUFORGEN Technical Bulletin, International Plant Genetic Resources Institute, Rome, Italy.

Lefèvre F., Kajba D., Heinze B., Rotach P., de Vries S. M. G. & Turok J. (2001b): Black poplar: a model for gene resource conservation in forest ecosystems. – Forestry Chronicle 77: 239–244.

Legionnet A. & Lefèvre F. (1996): Genetic variation of the riparian pioneer tree species *Populus nigra* L .1. Study of population structure based on isozymes. – Heredity 77: 629–637.

Lexer C., Buerkle C. A., Joseph J. A., Heinze B. & Fay M. F. (2007): Admixture in European *Populus* hybrid zones makes feasible the mapping of loci that contribute to reproductive isolation and trait differences. – Heredity 98: 74–84.

Lexer C., Fay M. F., Joseph J. A., Nica M.-S. & Heinze B. (2005): Barrier to gene flow between two ecologically divergent *Populus* species, *P. alba* (white poplar) and *P. tremula* (European aspen): the role of ecology and life history in gene introgression. – Mol. Ecol. 14: 1045–1057.

Meirmans P. G., Lamothe M., Perinet P. & Isabel N. (2007): Species-specific single nucleotide polymorphism markers for detecting hybridization and introgression in poplar. – Can. J. Bot. 85: 1082–1091.

Mejnartowicz M. (1991): Inheritance of chloroplast DNA in *Populus*. – Theor. Appl. Genet. 82: 477–480.

Pinon J. & Valadon A. (1997): Comportement des cultivars de peupliers commercialisables dans l'Union européenne vis-à-vis de quelques parasites majeurs. – Ann. Sci. Forestrières 54: 19–38.

Tuskan G. A., DiFazio S., Jansson S. et al. (2006): The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). – Science 313: 1596–1604.

Vanden Broeck A., Cox K., Quataert P., Van Bockstaele E. & Van Slycken J. (2003a): Flowering phenology of *Populus nigra* L., *P. nigra* cv. italica and *P.* x *canadensis* Moench. and the potential for natural hybridisation in Belgium. – Silvae Genetica 52: 280–283.

Vanden Broeck A., Quataert P., Van Slycken J., Roldán-Ruiz I. & Van Bockstaele E. (2003b): Pollen competition in *Populus nigra* females revealed by microsatellite markers. – Forest Genet. 10: 219–227.

Vanden Broeck A., Quataert P., Van Slycken J., Storme V., Boerjan W., Cottrell J. E. & Van Bockstaele E. (2004): Gene flow between cultivated poplars and native black poplar (*Populus nigra* L.): A case study along the river Meuse on the Dutch-Belgian border. – For. Ecol. Manage. 197: 307–310.

Van Loo M., Joseph J. A., Heinze B., Fay M. F. & Lexer C. (2008): Clonality and spatial genetic structure in *Populus ×canescens* and its sympatric backcross parent *P. alba* in a Central European hybrid zone. – New Phytol. 177: 506–516.

Vornam B., Herzog S., Preisig-Müller R. & Hattemer H. H. (1994): Restriction fragment length polymorphisms of a chloroplast photosystem II gene from poplar and their use for species identification. – Genome 37: 747–750.

Wolfe L. M., Blair A. C. & Penna B. M. (2007): Does intraspecific hybridization contribute to the evolution of invasiveness? An experimental test. – Biol. Inv. 9: 515–521.

Ziegenhagen B., Gneuss S., Rathmacher G., Leyer I., Bialozyt R., Heinze B. & Liepelt S. (2008): A fast and simple genetic survey reveals the spread of poplar hybrids at a natural Elbe river site. – Cons. Genet. 9: 373–379.

Zsuffa L. (1974): The genetics of *Populus nigra* L. – Annales Forestales (Zagreb) 6/2: 1–53.

Submitted 30 January 2008
Revision received 21 August 2008
Accepted 27 August 2008