Morphological study of the leaves of two European black poplar (*Populus nigra* L.) populations in Slovenia

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Abstract

Background and Purpose: *Conservation efforts across Europe and a substantial lack of information regarding the present status of black poplar* (Populus nigra L.) *in Slovenia led us to conduct this research. The objectives were to determine the presence of preserved native black poplar in Slovenia, to evaluate the variation within and between two selected populations, and to evaluate the condition of these populations, which is important for enabling their long-term gene pool conservation.*

Material and Methods: *To determine the black poplar distribution, the national database of growing stock of tree species in sub-compartments was used. Species determination was based on standard determination keys and descriptors. To assess a variation, a morphological comparison of leaves from two black poplar populations from the Sava and Mura rivers was done, as well as a comparison with a Canadian poplar* (Populus ×canadensis) *clone. Material for analyses was taken from 38 trees, totalling 3811 leaves. Descriptive statistics, non parametric Mann-Whitney U-tests, discriminant analysis (DA) and principal components analysis (PCA) were used for data analyses.*

Results and Conclusions: *The existence of well preserved European black poplar populations in Slovenia was confirmed. Sub-compartments that include black poplar in the growing stock cover 51 935.06 hectares, which represents 4.4% of the Slovenian forested area. Black poplar populations from the Sava and Mura sites differed significantly in morphological traits describing the lamina shape, while populations of black poplar and Canadian poplar differed mostly in petiole length and leaf apex shape. Variation within populations of black poplar was larger than between populations and variation of the Sava population was smaller than the Mura population. In the Mura population, only black poplar individuals were found, whereas 6 adult Canadian poplars of unknown origin were identified among black poplars in the Sava population. The recorded regeneration of black poplar was poor. Variation of the examined black poplar populations emphasizes the need to expand the research to other poplar populations in Slovenia and to start conservation activities as soon as possible.*

INTRODUCTION

European black poplar (*Populus nigra* L.) is a member of the *Salicaceae* family. It is a pioneer species with a short juvenile stage and rapid growth. It occurs on mainly alluvial plains where regeneration is

tightly connected to river floods and open areas with abundant light *(1)*. Because it is dioecious, out-crossing is necessary *(2)*, which results in high pollen migration and introduction of new genes *(3)*. Black poplar has wind pollinated flowers and some weeks after flowering a few million wind-distributed seeds *(4)* are scattered up to 3 kilometres *(5)*. Light seeds and wind distribution enable the exchange of seeds and genes between populations along river systems *(1)*, which enrich the population's genepool and, following local disturbances, together with vegetative shoots replace the lost population *(6)*. High levels of genetic diversity from sexual reproduction are crucial for the survival of the species in its dynamic habitats *(7)*.

Hybridization is common in the *Populus* genus. Poplars from the same section have the ability to hybridize, but sometimes poplars from different sections produce viable seeds as well *(8, 9)*. European black poplar (*Populus nigra* L.) and Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) belong to the section *Aigeiros*. The result of their genetic compatibility is the hybrid Canadian poplar (*Populus ×canadensis* Moench) *(10)* which has been widely planted across Europe for the needs of wood and paper industry. There is a concern of massive introgression of genes of foreign species into the native *P. nigra* genepool if cultivated F_1 hybrids backcross to native parental species *(2)*. Benetka *et al. (11)*, Fossati *et al. (7)* and Tabbener & Cottrell *(12)* could not confirm introgression of *P. deltoides* or *P. ×canadensis* into progeny of *P. nigra*, but Vanden Broeck *et al. (13)* and Ziegenhagen *et al. (14)* presented evidence for spontaneous backcrossing between female *P. nigra* and male *P. ×canadensis*. Once hybridization has begun, it is difficult to stop, especially if hybrids are fertile and produce progeny among themselves and with parental species *(15)*. Low levels of introgression from *P. ×canadensis* can be expected in natural populations of *P. nigra*, where no male black poplars are present around female trees and pollen of Canadian poplars can pollinate black poplar females *(2, 13)*. Introgression of *P. nigra* 'Italica' is also theoretically possible but has not yet been confirmed, possibly due to non-simultaneous flowering *(5, 12, 13)*. Flowering periods of poplars are extremely variable, not only between species or growing sites, but also between years *(16)*, and changes in flowering are not excluded in the coming decades of global climatic changes as well. Hybridization of *P. nigra* and other hybrids and clones reduces genetic variation, which together with habitat reduction can lead to the extinction of a rare plant species *(17)*.

In Europe, many studies have been done in order to determine the endangerment of *P. nigra (5, 7, 11, 12, 13, 14, 16, 18)*. Furthermore, much work has been done in the EU-funded project EUROPOP and the *Populus nigra* EUFORGEN Network *(2)*. In EUFORGEN Meeting Reports *(19)* many countries reported different states of black poplar, including significant reductions in populations or the complete disappearance of black poplar, the presence of very old solitary trees without any progeny, changed ecology of growing sites or excessive hybrid

plantations, while in some countries (i.e. Bulgaria, Croatia, Poland and Romania) very well preserved, isolated populations with active regeneration where rivers still flood have been reported. Black poplar is mostly threatened by heavy cuttings of native black poplar stands and planting poplar clones or hybrids on its natural growing sites, absence of regeneration, destruction and alteration of growing sites by human urbanization *(13, 16)*, changed dynamics of flooding due to hydraulic engineering followed by a lowering of the water table *(6)*, and natural hybridization and genetic pollution of black poplar's gene pool with alochtonous poplars grown in the nearby neighborhood *(1, 5)*. Lack of regeneration, an unbalanced sex ratio, high aged individuals, poor vitality, and small populations are important demographic indicators of the poor health condition of poplar stands *(20, 21)*. Conservation strategies are limited, and include *ex situ* strategies (i.e. nurseries and gene banks) and *in situ* strategies (i.e. restoration of natural habitats and protection of wider geographical units with preserved natural stands). Isolation from hybrids is especially needed *(1)*.

Black poplar grows throughout Slovenia, concentrated along main rivers and their tributaries *(22)*. Areas covered with natural poplar forests have been declining since the 1960s, when soil reclamation started along with intensive establishment of plantations of poplar clones and hybrids. Between the 1960s and 1980s, mostly the following clones of *P. ×canadensis* were tested and grown in Slovenia: 'I-154', 'I-214', 'I-264', 'I-455', 'I-476', 'I-488', '45/51', 'Marilandica' and 'Robusta' *(22)*. *Populus nigra* 'Italica' was widely used for wind stopping barriers and protection of river banks. Today poplars of various origins are used for recultivation of dumps and parking place greening. There are no specific conservation programs for black poplar in Slovenia. A general protection strategy is assured through national environmental and forest legislation. Future goals are oriented toward the classification of black poplar on the IUCN vulnerable species list and determination of pure black poplar stands appropriate for *in situ* conservation *(22)*.

There has not been much published information regarding the status of poplars in Slovenia after the 1990s, except by Božič *et al. (22)*. The fact remains that locations of hybrid planting sites and their condition are mostly unknown and there has been no evaluation of the danger for genetic pollution of native European black poplar in Slovenia. The goal of our study was to determine the distribution of poplars in Slovenian forests and to examine the presence of preserved native black poplar populations within them. Further goal was to evaluate the variation within and between two selected black poplar populations and to evaluate their condition, which is important for enabling their long-term gene pool preservation. We also wanted to examine to what extent leaf morphological traits can be used as a distinguishing trait between black and Canadian poplar.

MATERIAL AND METHODS

To determine the distribution of poplars in Slovenian forests, the Slovenia Forest Service database of growing stock of tree species in sub-compartments (year 2005, files odseki.dbf, drevna.dbf and razfaz.dbf) was analyzed. The total area of poplar distribution in Slovenia was calculated by adding up the surfaces of all sub-compartments where poplars were present in the growing stock regardless of its quantity. It should be noted that within the category »poplar« in the database, not only *Populus nigra* but also *Populus alba* L. and eventual hybrids are included.

For further analyses, we focused on two *Populus nigra* populations within the established distribution range in Slovenia: the first in the prealpine region along the Sava river near Ljubljana (between 46°07'15''/14°26'33'' and 46°05'01''/14°34'33'') and the second in the subpannonic region along the Mura river near Veržej (between 46°36'24''/16°08'17'' and 46°32'01''/16°17'34''). For comparison, we also examined one Canadian poplar (*Populus ×canadensis*, clone 'Imperial Carolina' ('DN-34')) population planted on a landfill near Ljubljana (46°01'35''/14°28'46'') (Figure 2). In the Sava, Mura and Canadian poplar populations, we randomly chose 16, 17 and 5 adult trees, respectively, and determined the species of each tree. The sample size of the Canadian poplar population was smaller because the sampled trees belong to the same clone and showed less variation than trees in other two populations.

For species determination, the descriptors from Lefévre & Kajba *(20)* and Kajba *et al*. *(23)*, identification keys of Martinčič *et al*. *(24)*, Fitschen *(25)*, Roloff & Bärtels *(26)* and the Identification sheet for *Populus nigra* *(27)* were used. A combination of several traits was used: shape and size of leaves, presence of leaf glands, number of parts that form seed capsules, and pattern on shoots.

To evaluate variation within and between the two selected *P. nigra* populations and to check if leaf morphological traits can be used as a distinguishing trait between black and Canadian poplar, morphological analyses were performed. In total 38 adult trees were used for leaf morphological assessment, and at least 100 fully developed leaves were sampled from each individual tree. Leaves were selected from the middle part of short shoots growing in a sunny position. All leaves were dried and herbarized. After herbarization they were scanned and measured with the software »WinFOLIA Pro 2005« of Regent Instruments Inc. In total 3811 leaves were analyzed. Descriptive statistics were used to describe the basic features of the data and non parametric Mann-Whitney U-tests were used to test the differences between two populations of black poplar and to test the differences between black and Canadian poplar. In addition, two different multivariate procedures were used to analyze the data: i) principal component analysis (PCA), where synthetic variables were linear combinations of original variables (mean values of 100 leaves per tree) that were constructed in a way that they displayed the largest variance be-

tween trees and ii) discriminant analysis, where the linear set of variables was used to distinguish between the Sava and Mura populations as well as between the black and Canadian poplar populations. All computations were performed with the software SPSS 15.0 for Windows.

For morphological analyses of leaves, the following traits were chosen according to previous research *(23)* and the software WinFOLIA (Figure 1):

- A – leaf area (cm^2),
- P – perimeter (cm),
- LeL – leaf length (cm),
- LW – lamina width (cm),
- LW/LL – lamina width/length ratio
- COEF – form coefficient ($4\pi A/P^2$)
- LL – lamina length (cm),
- dLW – distance from the lamina's base to the point where lamina width was measured, (cm)
- W80 – width of lamina on 80% of lamina's length from lamina's base up (cm),
- W90 – width of lamina on 90% of lamina's length from lamina's base up (cm),
- α10 - angle at 10% of lamina's length from lamina's base up (°),
- α25 - angle at 25% of lamina's length from lamina's base up (°),
- PL – petiole length (cm),
- α - angle between the midrib and the lowest lateral vein (°),
- PL/LeL – petiole length/leaf length ratio,
- dW80 – distance of leaf's apex to W80 (cm),
- dW90 – distance of leaf's apex to W90 (cm),
- W80/dW80 – ratio between W80 and dW80,
- W90/dW90 – ratio between W90 and dW90.

In addition, 3 research plots (100 × 25 m each) were set up within the Sava population in order to analyze the condition of the population important for enabling its long-term gene pool preservation. All 3 plots were placed in mature mixed stands, where *Populus nigra* was accompanied by *Salix eleagnos* Scop., *S. daphnoides* Vill., *S. alba* L., *Quercus robur* L., *Acer pseudoplatanus* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., *Carpinus betulus* L., *Robinia pseudoacacia* L., *Prunus avium* L., *Alnus glutinosa* (L.) Gaertn. and also *Picea abies* (L.) Karst. and *Pinus sylvestris* L. On each plot, species determination of all present individuals, assessment of the sex ratio, and measurement of the stand diameter structure and poplar regeneration were performed in 2006 and 2007.

TABLE 1

Means, standard deviations, minimum and maximum values of selected morphological traits. The U-test Sava/Mura was made between the Sava and Mura populations, while the U-test black/Can. was made between populations of black poplar (merged Sava and Mura) and Canadian poplar. Significant values of U-tests are given in bold (*: $p < 0.05$; **: $p < 0.01$). The number of leaves in the analysis: 3811.

Morphological traits	A			P			LeL			LW			LW/LL			COEF		
Populations	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.
Mean	28.43	28.17	28.95	23.90	24.97	26.57	13.01	13.30	14.66	6.54	6.36	6.33	0.82	0.77	0.74	0.62	0.57	0.52
SD	3.99	4.64	2.39	1.88	2.71	0.95	0.81	0.96	0.52	0.47	0.60	0.32	0.04	0.04	0.03	0.04	0.04	0.02
Min.	22.24	21.02	25.85	20.89	20.61	25.21	11.39	11.18	13.95	5.63	5.38	5.97	0.76	0.70	0.70	0.53	0.48	0.48
Max.	34.92	38.11	31.80	27.42	31.11	27.60	14.51	15.06	15.37	7.21	7.39	6.71	0.90	0.85	0.76	0.67	0.63	0.54
U-test Sava/Mura		0.692			0.235			0.331			0.331			**0.002**	**		**0.002**	**
U-test black/Can.		0.560			**0.023**	*		**0.003**	**		0.619			**0.012**	*		**0.002**	**

Morphological traits	LL			dLW			W80			W90			(α10)/2			(α25)/2		
Populations	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.
Mean	7.98	8.28	8.56	2.18	2.23	2.28	1.10	1.12	0.96	0.42	0.41	0.36	67.81	63.00	65.20	57.38	55.41	55.00
SD	0.57	0.58	0.26	0.15	0.22	0.05	0.16	0.25	0.05	0.06	0.09	0.02	3.31	7.05	2.86	1.54	1.73	1.22
Min.	6.99	7.16	8.13	1.87	1.93	2.21	0.74	0.81	0.90	0.30	0.29	0.34	60.00	47.00	62.00	54.00	52.00	53.00
Max.	8.90	9.59	8.81	2.46	2.64	2.35	1.39	1.92	1.04	0.51	0.68	0.39	72.00	72.00	69.00	59.00	58.00	56.00
U-test Sava/Mura		0.183			0.640			0.692			0.449			**0.029**	*		**0.003**	**
U-test black/Can.		0.080			0.136			**0.017**	*		**0.026**	*		0.487			0.101	

Morphological traits	PL			α			PL/LeL			W80/dW80			W90/dW90		
Populations	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.	Sava	Mura	Can.
Mean	5.03	5.03	6.11	50.16	45.78	50.04	0.39	0.38	0.41	0.70	0.68	0.56	0.54	0.51	0.43
SD	0.46	0.72	0.31	2.83	4.84	1.71	0.02	0.04	0.01	0.12	0.15	0.04	0.08	0.12	0.03
Min.	4.24	4.02	5.82	46.23	36.31	48.33	0.33	0.34	0.40	0.49	0.52	0.52	0.40	0.36	0.39
Max.	5.69	6.86	6.56	54.80	54.68	52.29	0.43	0.46	0.43	1.02	1.14	0.60	0.75	0.81	0.46
U-test Sava/Mura		0.719			**0.003**	**		0.056			0.517			0.249	
U-test black/Can.		**0.001**	**		0.252			**0.009**	**		**0.013**	*		**0.021**	*

RESULTS

Analyses of the database revealed that sub-compartments including poplars in the growing stock cover 51 935.06 hectares, which represents 4.4% of the Slovenian forested area. They mainly concentrate along the Mura, Drava, Sava, Krka and Soča rivers and their tributaries, but are also sporadically present in lowlands in other parts of Slovenia (Figure 2). *Populus nigra* is not specified as the only present poplar species in these sub-compartments (*P. alba* and poplar hybrids are included, too), but since both other taxa very often occupy similar sites and are relatively rare in Slovenia, we can roughly consider
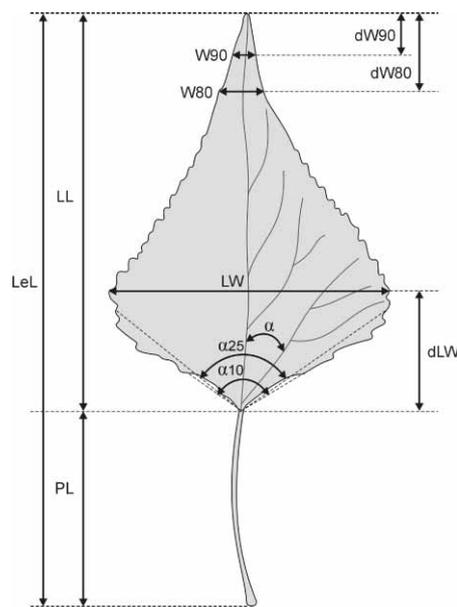
Figure 1. *Morphological traits used in analyses.*

this area as a wider distribution area of the black poplar in Slovenia.

With the use of identification keys and descriptors, in both Sava and Mura populations all 33 adult poplar trees which were randomly sampled for further morphological analyses were identified as *Populus nigra*. This clearly confirms the presence of well preserved native populations of this species.

Morphological analyses revealed that the mean values of lamina length, lamina width and petiole length of black poplar (Table 1) are consistent with values given in Fitschen *(25)* and Roloff & Bärtels *(26)*, where lamina length measures 5–12 cm, lamina width 4–10 cm, and petiole length 2–6 cm. Kajba *et al. (23)* report shorter and narrower leaves, similar angles from the midribs, and a larger distance from lamina's base to the widest lamina point (dLW). Mann-Whitney U-tests show that the Sava and Mura populations differ significantly in several morphological traits describing the lamina shape (Table 1),
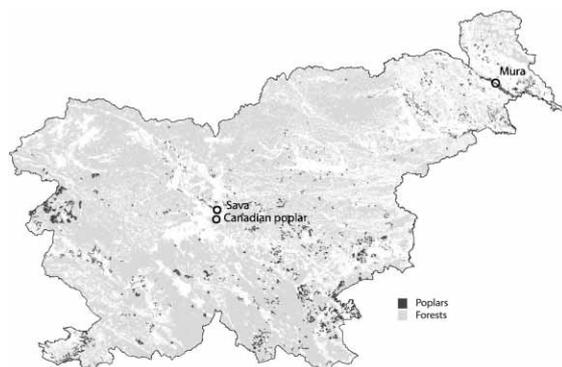

Figure 2. *Distribution of poplars (*P. nigra*, *P. alba *and hybrids) in the forest area of Slovenia and the location of studied populations.*

while populations of black and Canadian poplar differ mostly in petiole length and leaf apex shape.

No clear differentiation was found between black poplar populations and Canadian poplars with the PCA analysis (Figure 3). The first synthetic variable contributed to a major part of total variance (Table 2) as expected but, in addition, the next two synthetic variables showed a high contribution as well. The morphological traits contributing most to the first component were those describing leaf apex shape. Morphological traits that describe leaf size contribute most to the second component (Table 2).

Based on the results of discriminant analysis, the Sava and Mura populations cannot be reliably distinguished due to overlapping of characters (Figure 4). The first discriminant analysis conducted on the set of 33 specimens of black poplar correctly assigned 90.9% of specimens to the Sava and Mura subsets. Black poplar populations from the Sava and Mura sites are differentiated mostly by the morphological traits describing leaf lamina base and the leaf shape (COEF, LW/LL, $\alpha25$, α, $\alpha10$) (Table 3). All these traits are also the traits with the highest *F* values in univariate analysis. Overlapping of both populations is consistent with the fact that they both belong to the species *Populus nigra*. On the other hand, the second analysis on the entire 38 specimens correctly assigned 100% of specimens to black and Canadian poplar subsets (Figure 5). Black and Canadian poplar populations are clearly distinguished by the morphological traits describing leaf petiole length and leaf shape (Table 4).

In the three plots within the Sava population, all present poplars were identified as *Populus nigra* on research plots 1 and 2 (22 and 16 trees, respectively), while on research plot 3, based on morphological traits such as the cordate lamina's base and a hybrid-like undulate leaf margin, 6 adult poplars out of 41 were identified as Ca-
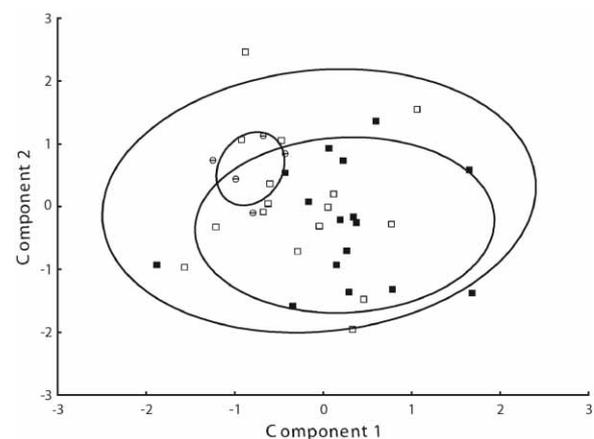

Figure 3. *PCA analysis: dispersion of individuals along the first and the second component. Ellipses delimit the space that includes, with $P_{0.95}$, the samples of Mura population (white squares, outer ellipse), Sava population (black squares, inner ellipse) and Canadian poplar population (white circles, the smallest ellipse).*

The first three components extracted by PCA with percentages of variance explained (per component) and the main morphological traits contributing to them.

	% variance	Main morphological traits
Component 1	33	W80, W90, W80/dW80, W90/dW90
Component 2	27	P, LL, A

nadian poplars (Table 5). On all three plots male trees were more frequent than female trees, and the 50:50 sex ratio among Canadian poplars on plot 3 indicates that those individuals do not belong to the same clone. On all three plots, regeneration was thoroughly checked and only on plot 3 progeny was found growing on a sunny location on the river bank. Five saplings, recognized as *Populus nigra*, were found on a 1m^2 area, all between 1 and 2 metres tall. Due to low understory light levels and competition from ground vegetation (*Fallopia japonica* Houtt. Ronse Decraene *Urtica* sp. and *Solidago* sp.) regeneration of other tree species was also infrequent. Only in plot 3 natural regeneration of *Fraxinus excelsior* and *Acer pseudoplatanus* was observed.

Stand structure is presented in Figure 6, classified by diameter classes. Plots 1 and 3 had trees prevailing in
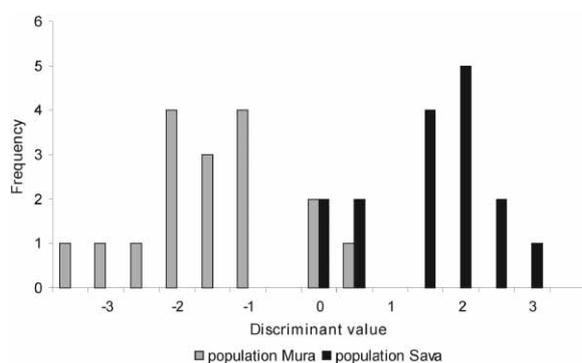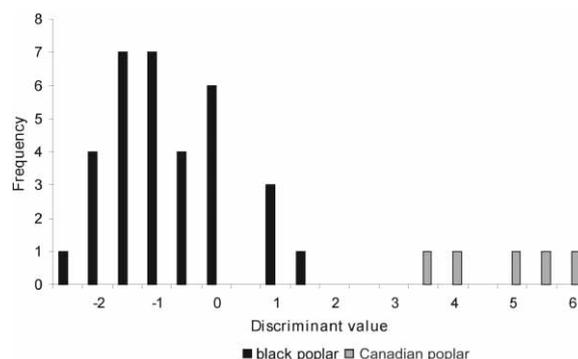

Figure 4. *Discriminant analysis based on 17 morphological leaf traits with the most influential morphological traits describing leaf shape and lamina base.*

TABLE 3

Standardized discriminant function coefficients for the five most correlated traits that separate Sava and Mura populations.

Variables	Function
form coefficient (COEF)	0.403
lamina width/length ratio (LW/LL)	0.390
angle at 25% of lamina's length (α25)	0.387
angle between the midrib and the lateral vein (α)	0.355
angle at 10% of lamina's length (α10)	0.280

Figure 5. *Discriminant analysis based on 17 morphological leaf traits with the most influential morphological traits describing leaf petiole and leaf shape. The Sava and Mura populations are merged as black poplar.*

class 21–30 cm, while plot 2 in the class over 40 cm. According to the stand diameter structure, we can conclude that stands on plots 1 and 3 were younger than that on plot 2.

DISCUSSION

Our results confirm the existence of well preserved European black poplar populations in Slovenia. On the whole, they are distributed over a relatively large part of Slovenia's forest area, but the present structure of the national database does not allow very firm conclusions about its precise distribution. Therefore, for a more reliable and accurate distribution picture of black poplar and for successful conservation of its genetic resources, the records in the database will have to be refined in the future. At present, only two groups of poplars are distinguished in the database: the first is aspen (*Populus tremula* L.), while in the second group under the name »poplar«, *Populus nigra*, *P. alba* and hybrid poplars are merged. The quality and applicability of a database should undoubtedly benefit greatly if these three species are recorded separately in future inventories.

Variation within populations of black poplar was larger than between populations and variation within the Sava population was smaller than within the Mura population. This might be a result of different ecological conditions in the compared areas. The difference could have arisen from diverse hydrological characteristics of

TABLE 4

Standardized discriminant function coefficients for the four most correlated traits that separate black and Canadian poplars.

Variables	Function
petiole length (PL)	0.336
form coefficient (COEF)	−0.293
petiole lenght/leaf length ratio (PL/LeL)	0.213
lamina width/ length ratio (LW/LL)	−0.207

Species share, sex ratio and the number of saplings on three plots in the Sava population.

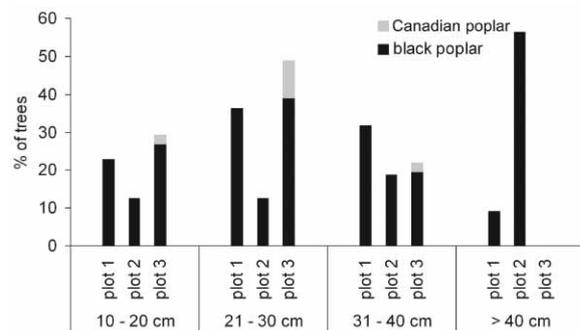|	Plot 1 n = 22		Plot 2 n = 16		Plot 3 n = 41	
	P. nigra	*P. ×canadensis*	*P. nigra*	*P. ×canadensis*	*P. nigra*	*P. ×canadensis*
No. of individuals	22	0	16	0	35	6
Sex ratio (male:female) (%)	68:32	/	56:44	/	83:17	50:50
Regeneration (the number of saplings)	0	0	0	0	5	0

Figure 6. *Percentage of trees in three research plots in Sava population classified by diameter class.*

rivers, such as river flow dynamics, which might have caused a larger range of growing conditions and higher within-population variation of the tested morphological traits along the Mura river. It is also possible that the Sava population possesses less genetic variation than the Mura population. Lower genetic variation can be a result of the fact that the Sava population originates from a part of a river not very distant from the river source in the Julian Alps. The Sava river basin upstream from Ljubljana is relatively small, for the most part alpine, and provides only limited areas with favorable natural conditions for black poplar, mainly concentrated in a narrow belt along the river, and similar conditions prevail along most of the downstream portion of the river in Slovenia. As a result, the Sava population is considerably smaller and more isolated than the Mura population. Lower variation of the Sava population might thus be a result of a stronger isolation or genetic drift, or possibly a result of vegetative origin after a massive flood in the past. On the other hand, the Mura river basin is considerably larger and includes more extended natural black poplar populations growing in a wider range of different sites along the river and its tributaries. In black poplar, a genepool is mostly enriched through bidirectional wind dispersal of pollen and seeds along riverbanks (1), but additional downstream gene flow through vegetative parts as well as seeds along the Mura river may have also contributed to its larger diversity.

There were significant differences between morphological traits of the Sava and Mura populations, but none of them were strong enough to distinguish between populations. PCA analysis showed little dispersion of the hybrid Canadian poplar trees, consistently for all 5 trees belonging to the same clone 'Imperial Carolina'.

Morphological variation within *Populus nigra* and between *P. nigra* and the Canadian poplar *P. ×canadensis* 'Imperial Carolina' was detected by all analyses, mainly determined by morphological traits of lamina shape and petiole length. For differentiation of the Sava and Mura populations, U-tests and discriminant analysis showed significant differences in morphological traits describing the lamina shape. For Canadian poplar identification, usually a combination of morphological traits, such as undulate leaf margin with some permanent or temporary hairs along it, the presence of 1–2 leaf glands, more than 2 parts forming a seed capsule, triangular leaf shape and a petiole length above 6 cm, are used *(25, 26, 27)*. When leaves of black and Canadian poplar were compared in our research, morphological traits describing leaf apex shape and petiole length differed significantly. Leaves of black poplar were slightly more long-pointed compared with the Canadian poplar. The analyses of the petiole length as an easily measurable quantitative trait revealed that petioles of the Sava and Mura black poplar populations were shorter than 6 cm, whereas Canadian poplars were longer than 6 cm (Table 1), suggesting that the petiole length might indeed be used as an identification trait between *P. nigra* and *P. canadensis* 'Imperial Carolina' and possibly between *P. nigra* and other Canadian poplar clones, too. However, it should be emphasized that these are the mean values and in practice a larger sample improves the reliability of a distinction.

The stand structure of the Sava black poplar population comprised abundant large (older) trees, especially in plot 2, and small diameter (younger) trees on plots 1 and 3. In none of the plots, trees less than 10 cm in diameter were found, except for 5 saplings in plot 3. Trees in smaller size classes are consistent with observations on plots: the underground water level on plots 1 and 3 was higher, trees on both plots grew closer to the river bank and river embankments were less steep and changed. As indicated in an earlier research, sex ratio problems in populations can be related either with too few males in stands (e.g. only 2 males on 100 females; *21*) or too large distances between males and females (e.g. 25 km; *13*). Our results on the one hand show an adequate sex ratio to enable successful fertilization and seed production in the Sava population, but on the other hand there is an obvious lack of favorable germination conditions. Regen-

eration in most stands is absent or poor and only five sap-lings have been found in plot 3. Their origin is unknown, but since they grow on a very limited surface and very close to each other, their possible vegetative origin cannot be excluded. Our observation of poor regeneration in old stands confirms what has been found in other research, too *(1)*. Likely causes of the absence of regeneration may be shading due to canopy and ground vegetation layer, lack of fresh sandy sediments, low vitality of mature trees and drying of the growing sites due to lowering of the water level. We can conclude that a satisfactory sex ratio in combination with favorable germination places could be important for the long-term conservation of native black poplar populations in Slovenia.

The results of this study showed that pure popula-tions of black poplar on the Sava and Mura rivers are still preserved, despite intensive cultivation of hybrids in the past in some areas. Based on morphological traits, no hy-bridization or introgression of hybrids into native black poplar populations was detected, but it is known that identification of hybrid poplars using only morphologi-cal traits can be unreliable and problematic, especially when hybrids are in the progeny *(11, 15, 28)*. In the Mura population no hybrid trees were found, while on one plot in the Sava population 6 adult trees of unknown origin were identified as Canadian poplars. They do not seem to be a result of direct planting on the site, so they might have become established via incidental Canadian poplar seed or vegetative dispersal from an older plantation. However, even if not very likely, some of them might be a direct result of the introgression on the site. Therefore, for more reliable conclusions about their origin addi-tional research using molecular methods needs to be per-formed. Additional populations with larger sample sizes as well as the application of molecular methods will be needed in the future to investigate the extent of the introgression of Canadian poplar genes to native black poplar populations. The future of black poplar in Slo-venia depends on spreading urbanization and success of environmental conservation. Unpredictable climatic chan-ge with occasional vast floods can improve regeneration (new sedimentation, removal of old trees) or on the other hand can cause even drier conditions in black poplar habitats. The condition and wide variation of the exam-ined black poplar populations clearly demonstrate the need to expand the research to other poplar populations in Slovenia and to start conservation activities as soon as possible. When choosing the future gene conservation units of black poplar we should focus on the areas where the species grows vigorously, where river dynamics and regeneration are active, and where the isolation from hy-brids can be attained.

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