

## Introgressive hybridization between Scots pine and mountain dwarf pine at two localities of northern Slovakia

Andrej Kormuťák<sup>1,2</sup>, Vladimír Čamek<sup>1</sup>, Martina Branná<sup>2</sup>, Martina Čelková<sup>2</sup>, Božena Vooková<sup>1</sup>, Peter Maňka<sup>3</sup>, Martin Galgóci<sup>3</sup>, Peter Boleček<sup>2</sup>, Dušan Gömöry<sup>4</sup>

<sup>1</sup>Institute of Plant Genetics and Biotechnology SAS, Akademická 2, P. O. Box 39A, SK-950 07 Nitra, Slovak Republic, e-mail: nrgkorm@savba.sk

<sup>2</sup>Department of Botany and Genetics Faculty of Natural Sciences, Constantine Philosopher University, A. Hlinku 1, SK-949 74 Nitra, Slovak Republic

<sup>3</sup>Arboretum Mlyňany SAS, Vieska nad Žitavou 178, SK-951 52 Slepčany, Slovak Republic

<sup>4</sup>Faculty of Forestry, Technical University in Zvolen, T. G. Masaryka 24, SK-960 53 Zvolen, Slovak Republic

### Abstract

KORMUŤÁK, A., ČAMEK, V., BRANNÁ, M., ČELKOVÁ, M., VOOKOVÁ, B., MAŇKA, P., GALGÓCI, M., BOLEČEK, P., GÖMÖRY, D. 2013. Introgressive hybridization between Scots pine and mountain dwarf pine at two localities of northern Slovakia. *Folia oecol.*, 40: 201–205.

Introgressive hybridization within hybrid swarm populations of *Pinus sylvestris* and *P. mugo* was analyzed in the localities Medzi Bormi and Sokolie, northern Slovakia, using species-specific *trnV-trnH/Hinf*I restriction profiles of chloroplast DNA of needles and zygotic embryos. The presence of trees with both *P. sylvestris* and *P. mugo* haplotypes indicates hybrid nature of the swarms. Molecular analysis revealed a relatively high rate of introgressive hybridization between putatively hybrid swarm individuals and the parental trees of the pure species *P. sylvestris* and *P. mugo* growing in the same localities. The proportion of hybrid seeds with *P. sylvestris* pollen donor averaged at 30.3%, whereas those with pollen donor of *P. mugo* at 19.8%. The results are discussed within context of the established crossability relationship between *P. sylvestris* and *P. mugo*.

### Keywords

introgressive hybridization, *P. mugo*, *Pinus sylvestris*

### Introduction

Scots pine and mountain dwarf pine represent the two principal species of pines indigenous to Slovakia with predominantly allopatric distribution in the country. The altitudinal range of the former extends from the lowlands of 200 m a.s.l. up to 1,465 m (KRIPPEL, 1986), whereas that of the latter between 1,400 m and 1,800 m (SOMORA, 1981). However, in some places of northern Slovakia the natural habitats of these species overlap creating conditions for their spontaneous hybridization. The hybrid swarms generated in this way involve the hybrid individuals along with the trees of Scots and mountain dwarf pines. The hybrid nature of these swarms exerts profound effect on reproductive behaviour of their trees. Except for hybridization of hybrid individuals with each other, the back-crossing of the

hybrids with one or both parental species is supposed to take place in a variable extent. The process is called introgressive hybridization and is believed to contribute substantially to the increased variability in participating species (ANDERSON, 1949).

In present study, we followed this process in two hybrid swarm populations of Scots pine and mountain dwarf pine in northern Slovakia using DNA molecular markers.

### Material and methods

#### Species and populations

The hybrid swarm population of Scots pine (*Pinus sylvestris* L.) and mountain dwarf pine (*P. mugo* Turra)

growing in the peat-bog locality „Medzi Bormi“ near Zuberec (alt. 815 m) together with the hybrid swarm growing on calcareous rocks of the locality „Sokolie“ near Terchová (alt. 1,172 m) were subjected to molecular analysis of introgressive hybridization. As a control, the pure species populations of *P. mugo* in Roháče (alt. 1,500 m) and *P. sylvestris* in Hruštín (alt. 800 m) were used.

The principle according to which introgressive hybridization assessment was made involved comparison of the chloroplast DNA (cpDNA) haplotypes of both needles and zygotic embryos of a given tree. Owing to the paternal inheritance of cpDNA in conifers (WAGNER et al., 1987), the identical haplotype of the maternal tree (haplotype *P. sylvestris*) and its zygotic embryo (haplotype *P. sylvestris*) was taken for intraspecific crossing. On the contrary, the opposite haplotypes of the maternal tree (haplotype *P. sylvestris*) and its embryo (haplotype *P. mugo*) was taken as an evidence for introgressive hybridization between hybrid individual of *P. sylvestris* haplotype and *P. mugo*. Molecular analysis of the hybrid swarms Medzi Bormi a Sokolie was carried out twice, in the years 2009 and 2010 involving 23 trees in the locality Medzi Bormi in 2009 and 10 trees in 2010. At the locality Sokolie, 20 trees were subjected to analysis in 2009 and 19 trees in 2010. Control populations of *P. mugo* and *P. sylvestris* were represented in the experiment with 10 trees each subjected to analysis in 2009 only. The extent of experiment performed with hybrid swarm populations depended on the number of trees bearing cones in a given year.

### DNA extraction

Total DNA was separately extracted from needles of individual trees using CTAB method by MURRAY and THOMPSON (1980). The number of seeds from corresponding trees which were involved into study ranged between 9 and 10 sound seeds. In *P. sylvestris* from Hruštín 15 seeds per tree were used.

Following a 3–5 day imbibition of seeds on wet paper wool in Petri dishes, the embryos of individual seeds were excised from surrounding female gametophyte tissue and used in DNA extraction. Each embryo was extracted in Eppendorf tube using rotatory homogenizer with a pestle. More advanced germinating embryos were extracted with NucleoSpin Plant Kit (Macherey-Nagel), those which had not protruded from the seed coat with Simax™ Genomic DNA Extraction Kit (Beijing SBS Genetech Co., Ltd.).

### PCR-RFLP analysis

The *trnV-trnH* region of cpDNA was PCR amplified as described earlier (KORMUTÁK et al., 2008). The obtained PCR products were digested with restriction enzyme *Hinf*I, which was found to discriminate the cpDNA of

*P. sylvestris* from that of *P. mugo* (WACHOWIAK et al., 2006a). The generated fragments were electrophoretically separated in 2 % agarose gels with EtBr and 1× TBE buffer.

### Results

Amplification of cpDNA *trnV-trnH* region of needles and embryos resulted in PCR-product of approximate size of 2,300 bp in both species (Fig. 1, lane-a). Its digestion with restriction enzyme *Hinf*I generated four fragments in *P. sylvestris* needles and three fragments in *P. mugo* needles, respectively (Fig. 1, lanes b–h; Fig. 2). No individual variation in restriction profiles of *P. sylvestris* and *P. mugo* was observed.

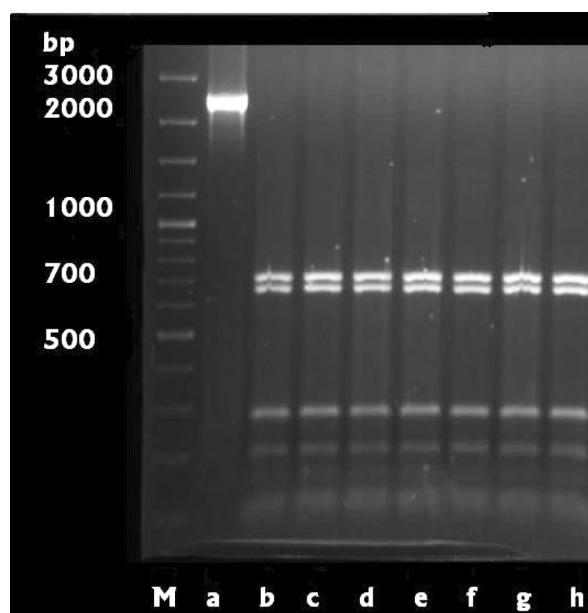


Fig. 1. PCR-product of *trnV-trnH* gene region of cpDNA (lane a) and its restriction fragments generated by *Hinf*I in *P. sylvestris* individuals (lanes b–h); M, molecular size marker.

This indicates necessarily the species-specific nature of the differences scored in these species. Of particular importance there was in this respect the DNA fragment of 700 bp size characteristic for *P. mugo* along with the 680 bp size fragment characteristic for *P. sylvestris* which have enabled to discriminate reliably between the parental species and to score efficiently the gene flow between the trees of hybrid swarms. The results of this study are summarized in Table 1.

As expected, all the trees of *P. sylvestris* in Hruštín shared *P. sylvestris* haplotype and the same was true of their embryos which exclusively exhibited *P. sylvestris* haplotype. Similar figure was characteristic for *P. mugo* population in Roháče with *P. mugo* haplotype shared uniformly by the scored trees and their embryos.

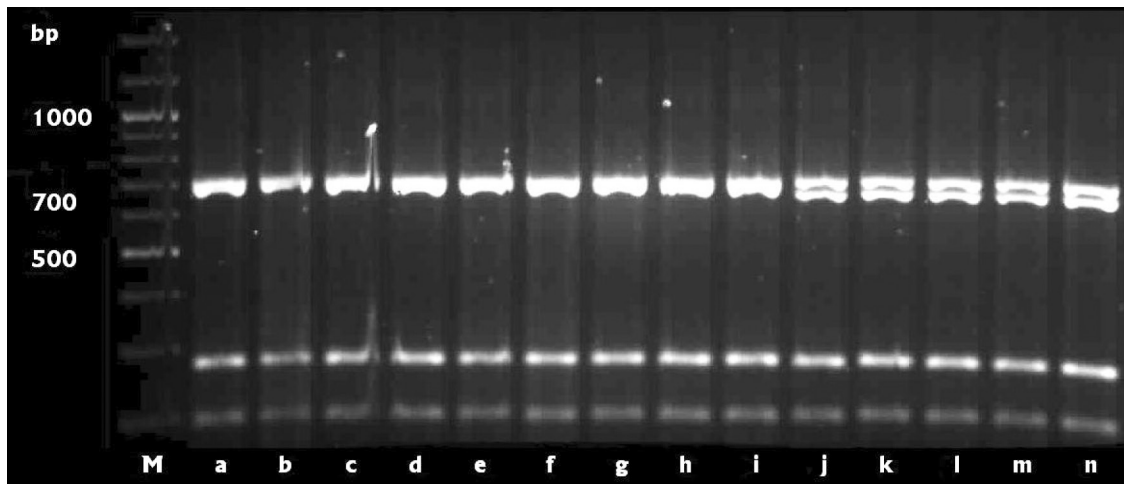


Fig. 2. *trnV-trnH/HinfI* restriction profiles of eight *P. mugo* individuals (lanes a–i) and five *P. sylvestris* individuals (lanes j–n); M, molecular size marker.

Table 1. Frequency occurrence of introgressive hybrids in localities Medzi Bormi and Sokolie in 2009 and 2010

Locality	Year	Individuals		Number of anal. seeds	Embryo haplotype		Hybrid seeds [%]
		Haplotype	Numb.		<i>P. sylvestris</i>	<i>P. mugo</i>	
Hruštín	2009	<i>P. sylvestris</i>	10	153	153	0	0
Roháče	2009	<i>P. mugo</i>	10	100	0	100	0
Medzi Bormi	2009	<i>P. sylvestris</i>	16	170	123	47	27.6
		<i>P. mugo</i>	7	76	41	35	53.9
	2010	<i>P. sylvestris</i>	2	20	11	9	45.0
		<i>P. mugo</i>	8	80	9	71	11.2
Sokolie	2009	<i>P. sylvestris</i>	8	84	56	28	33.3
		<i>P. mugo</i>	12	114	32	82	28.0
	2010	<i>P. sylvestris</i>	18	175	170	5	2.8
		<i>P. mugo</i>	1	10	3	7	30.0

Contrary to the control populations mentioned above, the hybrid swarm populations are much more heterogeneous involving the trees of both *P. sylvestris* and *P. mugo* haplotypes. As it follows from Table 1, their proportions varied not only in the tested localities but also annually in a given locality. Contrasting differences in proportions of the two haplotypes in 2009 and 2010 in the localities Medzi Bormi a Sokolie were due to the preferable sampling of those trees which bore mature cones in the respective years. For example, there were 8 trees of *P. sylvestris* haplotype and 12 trees of *P. mugo* haplotype on Sokolie scored in 2009 but the next year the reverse figure was obtained in the locality involving 18 trees of *P. sylvestris* haplotype and 1 tree of *P. mugo* haplotype only.

Considerably variable was also the rate of introgression on individual localities, including annual variation. Among 170 embryos scored in 2009 in 16 trees of *P. sylvestris* haplotype in the locality Medzi Bormi, 123

embryos were found to originate from pollination with *P. sylvestris* pollen and 47 embryos from pollination with *P. mugo* pollen. The amount of hybrid embryos *P. sylvestris* × *P. mugo* has accordingly attained the level of 27.6%. Even higher was the rate of introgression between the trees of *P. mugo* haplotype in the locality with *P. sylvestris* pollen, as evidenced by the 53.9% proportion of hybrid embryos *P. mugo* × *P. sylvestris* in 2009. The reverse situation was ascertained in 2010 with 45% proportion of *P. sylvestris* × *P. mugo* hybrid embryos and 11.25% share of the embryos of reciprocal combination detected in the same locality. Though lower, the rate of introgression in the locality Sokolie has exhibited the same tendency as described in the locality Medzi Bormi, with profound differences in proportions of *P. sylvestris* × *P. mugo* and *P. mugo* × *p. sylvestris* embryos in individual years (Table 1).

Summarily, the rate of introgression between the putative hybrid individuals of both localities investigat-

ed and the parental species *P. sylvestris* and *P. mugo* differs considerably. Among the total number of 449 seeds originating from 44 individuals of *P. sylvestris* haplotype, 89 hybrid embryos of *P. sylvestris* × *P. mugo* were detected representing 19.8% share. On the contrary, the proportion of hybrid embryos *P. mugo* × *P. sylvestris* averaged at 30.3%, based on 85 hybrid embryos revealed among 280 seeds from 28 putative hybrid individuals of *P. mugo* haplotype. The obtained data indicate different intensities in gene flow between individuals of the putative hybrid swarms of *P. sylvestris* and *P. mugo* on one hand and respective parental species on the other hand.

## Discussion

Of the two hybrid swarm populations investigated, only population Medzi Bormi was subjected to a detailed morphometric analysis. Based on needle morphology and needle anatomical traits, the hybrid nature of the population was postulated by MUSIL (1975) and VIEWEGH (1981). In case of the population Sokolie only indirect evidence of its hybridity has recently been provided involving intermediate habitus of the trees, reduced size of the cones and reduced number of seeds per cone (KORMUŤÁK et al., 2009). All these characteristics are also shared by the three additional hybrid swarms in northern Slovakia, i. e. those in Tisovnica, Suchá Hora and Medzi Bormi (KORMUŤÁK et al., 2011). Homogeneous nature of the controlled populations uniformly represented by the *P. sylvestris* haplotype in Hruštín and/or by the *P. mugo* haplotype in Roháče along with a heterogeneous nature of investigated hybrid swarm populations which involve the trees of both the above mentioned haplotypes may be taken for a strong hint indicating hybridity of the swarms. It is not clear whether tested trees of the hybrid swarms represent  $F_1$  generation of the true hybrids between *P. sylvestris* and *P. mugo* or if they are back-crosses of the  $F_1$  generation individuals with the parental species already. However, their ability to intercross with *P. sylvestris* and *P. mugo* provides a strong support for introgressive hybridization taking place in both investigated localities with hybrid swarm populations. It follows from the data obtained so far that hybrid swarm individuals intercross more efficiently with *P. sylvestris* than with *P. mugo*. This finding is in contradiction with the results of artificial hybridization between *P. sylvestris* and *P. mugo* carried out by WACHOWIAK et al. (2006b) as well as with the data of the authors referring to the lack of evidence for the hybrid swarm in sympatric population of *P. mugo* and *P. sylvestris* in the locality „Bór na Czerwonym“ at the northern foothills of the Tatra Mountains in Poland. In both cases the authors observed a rare hybridization between the parental species with only unidirectional gene flow from *P. mugo* to *P. sylvestris*. The reciprocal crosses *P. mugo* × *P. sylvestris*

have not been identified so far. The only explanation for a relatively high hybridological affinity between hybrid swarm individuals in the tested localities and the parental species *P. sylvestris* and *P. mugo* is a weakening of reproductive barriers between participating individuals due to the hybrid nature of the involved hybrid swarm individuals. However, this assumption needs to be verified further with the emphasis on proving hybridity of the hybrid swarm individuals.

## Acknowledgement

This study was financially supported by the VEGA Grant Agency, project no. 2/0076/09.

## References

- ANDERSON, E. 1949. *Introgressive hybridization*. New York-London: John Wiley and Sons. 109 p.
- KORMUŤÁK, A., MAŇKA, P., VOOKOVÁ, B., SALAJ, T., ČAMEK, V., BOLEČEK, P., GÖMÖRY, D. 2009. Seed quality in hybrid swarm populations of *Pinus mugo* Turra and *P. sylvestris* L. *Pl. Syst. and Evol.*, 277: 245–250.
- KORMUŤÁK, A., VOOKOVÁ, B., BRANNÁ, M., MAŇKA, P., GÖMÖRY, D. 2011. Cone size in putative hybrid swarm populations of *Pinus sylvestris* L. and *Pinus mugo* Turra in northern Slovakia. *Thaiszia – J. Bot., Košice*, 21: 161–165.
- KRIPEL, E. 1986. *Postglaciálny vývoj vegetácie Slovenska* [Postglacial development of the vegetation in Slovakia]. Bratislava: Veda. 307 p.
- MURRAY, M.G., THOMPSON, W.F. 1980. Rapid isolation of high molecular weight DNA. *Nucl. Acid Res.*, 8: 4231–4235.
- MUSIL, I. 1975. Príspevek k variabilite niekterých populácií komplexu *Pinus mugo* s. l. na Slovensku [Contribution to variability of some populations of the *Pinus mugo* complex s. l. in Slovakia]. In *XII. Vedecká konferencia o premenlivosti drevín Karpatského oblúka. Podbanské*, 3.
- SOMORA, J. 1981. Kosodrevina (*Pinus mugo* TURRA) v Tatranskom národnom parku (rozšírenie, vzrastové formy) [Mountain dwarf pine (*Pinus mugo* TURRA) in the Tatra National Park (distribution, growing forms)]. In *Zborník prác o Tatranskom národnom parku* 22. Martin: Osveta, p. 31–76.
- VIEWEGH, J. 1981. Variabilita hybridního roje *Pinus mugo* × *Pinus sylvestris* na rašeliníšti u Zuberce na Oravě [Variability of the *Pinus mugo* × *Pinus sylvestris* hybrid swarm on the peat-bog in Zuberec of the Orava region]. *Folia dendrol.*, 8: 41–62.
- WACHOWIAK, W., ODRZYKOSKI, I., MYCZKO, L., PRUS-GŁOWACKI, W. 2006a. Lack of evidence on hybrid swarm in the sympatric population of *Pinus mugo* and *Pinus sylvestris*. *Flora*, 201: 307–316.

- WACHOWIAK, W., STEPHAN, B.R., SCHULZE, I., PRUS-GŁOWACKI, W., ZIEGENHAGEN, B. 2006b. A critical evaluation of reproductive barriers between closely related species using DNA markers – a case study in Pinus. *Pl. Syst. and Evol.*, 257: 1–8.
- WAGNER, D.B., FURNIER, G.R., SAGHAI-MAROOF, M.A., WILLIAMS, S.M., DANKI, B. P., ALLARD, R.W. 1987. Chloroplast DNA polymorphism in lodgepole pines and jack pines and their hybrids. *Proc. Nat. Acad. Sci. U.S.A.*, 84: 2097–2100.

*Received December 6, 2012*

*Accepted March 26, 2013*