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Genetic Variation of *Pinus cembra* L. in the Ukrainian Carpathians by Microsatellite Loci

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Swiss stone pine, *Pinus cembra* L., is one a key coniferous species forming the timberline forest stands in parts of the European Alps and the Carpathians. The "island" characteristics of the alpine zone in the Carpathian Mountains and chronic deforestation foster range fragmentation of *P. cembra*, especially in Ukraine at the northeastern border of the species' range. Thus, restricted gene flow may affect the genetic structure of *P. cembra* populations through increased inbreeding. Our previous studies based on allozyme markers showed that *P. cembra* samples from the Ukrainian Carpathians and the Alps are characterized by lower levels of genetic variation and higher levels of inbreeding than the samples of the related stone pine species *P. sibirica, P. koraiensis* and *P. pumila*. The Carpathian populations of *P. cembra* revealed higher levels of expected heterozygosity and differentiation in comparison with Alpine samples, associated to range fragmentation of the species in this region (Belokon *et al.* 2005, Politov and Krutovskii 2004, Politov *et al.* 1992).

Recently, the analysis of chloroplast microsatellites (or single sequence repeats, SSRs) in *P. cembra* of the Northern and Southern Carpathians and the Tatra Mountains have revealed high haplotypic variation and large among-population variation (Höhn *et al.* 2005, Höhn *et al.*, in press). The present paper reports first results on the variation of nuclear SSRs of *P. cembra* in the Ukrainian Carpathians. We used three samples (Gadzhina, Yayko and Gorgany) presented by 27, 42, 43 individuals correspondently and 23 trees of *P. sibirica* in a provenance trial from Western Siberia (Napas). First, we tested 10 primer pairs initially developed for the related species, *Pinus strobus* (Echt *et al.* 1996). Due to inconsistent PCR product amplification or low polymorphism these primers were turned out to be hardly useful for *P. cembra* analysis. Among eight primers designed for *P. cembra* (Salzer *et al.*, in press), three loci were selected for further work. We combined the amplification of two loci, *Pc 18* and *Pc 23*, in one multiplex PCRn. Loci *Pc 1b* and *Pc 18* were multiallelic and highly polymorphic (Table 1). The studied sample of *P. sibirica* was characterized by lower allele number than *P. cembra* by *Pc 1b* and *Pc 23* loci. We observed the differences in the allele frequencies as well as the specific allele composition between populations and species.

The level of microsatellite variation in the studied populations was higher than it was earlier estimated by allozyme loci (Belokon *et al.* 2005, Politov and Krutovskii 2004). The values of mean observed (H_O) and expected (H_E) heterozygosities of *P. cembra* were H_O=0.435±0.104, H_E=0.684±0.159. The respective values for *P. sibirica* were higher (H_O=0.558±0.072, H_E= 0.749±0.063) than in *P. cembra*, as it was also observed in allozyme studies. The samples of *P. cembra* and *P. sibirica* were characterized by high levels of inbreeding (F_{IS}=0.368 and 0.238, respectively).

The analysis of molecular variance (AMOVA) showed that 10% of the total genetic variance was distributed among species, 5 % among populations and 85% within populations. The level of among-population differentiation (F_{ST}) and Nei's genetic distances (D_N) were lowest within the Carpathian samples of *P. cembra* (F_{ST}=0.046, D_N=0.069–0.195) and largest among all samples of *P. cembra* and *P. sibirica* (F_{ST}=0.078, D_N=0.386–0.393). The Principal Coordinates analysis showed

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Table 1. Nuclear microsatellite loci, primer sequences, allele size ranges and numbers in Carpathian samples of *Pinus cembra* and in samples of a *P. sibirica* provenance trial

Locus	Primer sequences (5'- 3')	Motif	*P. cembra**		*P. sibirica**	
			Allele size range	N_A	Allele size range	N_A
Pc 1b	F: CCACCATCTTGTTTTTGTGTTC R: TTCTCTCCACCCAGCCTAAA	$(GT)_{19}$	166–208 (166–202)	12 (10)	178–210 (174–212)	9 (9)
Pc 23	F: GGGCATCATTATTTTCTTACAA R: CTTGATATACCATGCCACAACC	$(TG)_6$ $CG(TG)_2$	206–260 (221–257)	10 (12)	205–235 (200–238)	8 (4)
Pc 18	F: TTCCCAAAGACCATAGAACCA R: TCATGAAATATTACGTCCCTTATCC	$(TG)_{12}$	154–162 (152–156)	4 (3)	154–162 (152–158)	4 (4)

Note: N_A – number of alleles, * - data from Salzer *et al.* (in press) are given in parentheses.

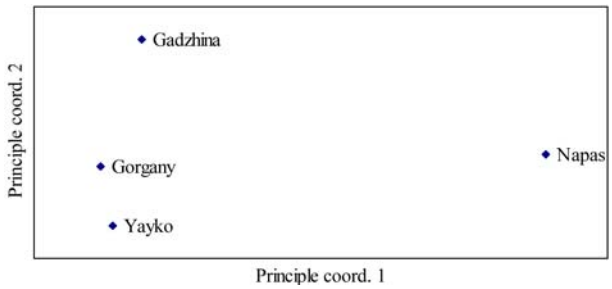

Figure 1. Principle coordinate analysis of *P. cembra and P. sibirica* samples based on three SSR loci.

a good correspondence between genetic differentiation and geographic origin of the studied samples of *P. cembra* and *P. sibirica* (Figure 1).

The data retrieved from nuclear SSR loci complement the earlier allozyme data of *P. cembra* genetic variation. The among-population differences in allele composition and frequencies could be applied for the identification of populations of *P. cembra* and for elaborating gene resources conservation strategy for this species vulnerable in the Carpathians.

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