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RAPD Analysis of Genetic Structure in Four Natural Populations of *Taxus baccata* from Southern Poland

MARCIN ZAREK

Department of Forest Pathology, Agricultural University of Cracow
al. 29 Listopada 46, 31–425 Cracow, Poland

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This work assessed genetic diversity and genetic structure using random amplified polymorphic DNA (RAPD) variation in 120 individuals of four natural populations of *Taxus baccata* growing in southern Poland (3 in mountains and one in lowland) to obtain basic information on this natural resource. With 9 primers, 185 highly reproducible and clear RAPD bands were obtained. Genetic diversity within populations was relatively high, with percentages of polymorphic bands ranging from 48.65% to 77.30%, averaging 69.59% (Shannon index 0.311). Global AMOVA showed that genetic variation between populations accounted for 26% of total variation, with the remainder (74%) occurring within population. Pairwise Φ_{PT} values were not correlated with geographic distance. Two groups of populations were distinguished by ANOVA and principal coordinate analysis (PCO) based on a Euclidean metric: those growing in mountains (Nowa Wieś, Cisowa Góra, Zadni Gaj), with higher internal diversity, and those growing in lowlands (Liswarta), with lower internal diversity. The results are typical for an outcrossing, wind-pollinated and long-lived woody species.

Key words: DNA markers, genetic diversity, RAPD, *Taxus baccata* L., yew.

INTRODUCTION

Taxus baccata L. is an evergreen needle-leaved gymnosperm shrub or tree, growing up to 28 m high. The species is slow-growing and long-lived, reaching maturity only at ~70 years. It is extremely shade-tolerant but can withstand full exposure to the sun. *Taxus* is normally dioecious, rarely monoecious, and wind-pollinated (Thomas and Polwart, 2003). Its seeds, surrounded by a red fleshy arillus, are dispersed endozoochorously by birds or small mammals; clonal regeneration is important but is restricted mainly to polycormic propagation (Hulme, 1996; Thomas and Polwart, 2003; Hilfiker et al., 2004).

English yew occurs all over Europe. In Scandinavia it grows up to 61°N latitude. The eastern limit of the range in Europe extends from the Gulf of Riga through the Białowieża Forest and down to the Carpathians. In the Alps it reaches 1100–1400 m a.s.l., in the Carpathians 1660 m, in the Caucasus 1500 m and in the Middle East 2300 m. It also grows in North Africa (Bugała, 1975).

The reason for *Taxus baccata*'s decline has been analyzed in many papers (e.g., Bugała, 1975; Giertych, 2000). Some authors try to connect it with suspected low genetic diversity, but yew's high adaptability to different habitat conditions, and the production of numerous ornamental varieties, testifies rather to high genetic diversity (Bugała, 1975; Lewandowski et al., 1995).

Until the 1980s, morphological markers were the only way to investigate variability (Elves and Henry, 1906; Pilger, 1916; Stecki and Bella, 1931; Krussmann, 1972; Kuświk, 1987). Significant progress in variability research took place with the discovery of molecular markers. For example, isoenzymatic markers have been used in research by Greer et al. (1993), Lewandowski et al. (1995), Wheeler et al. (1995), Hertel and Kohlstock (1996), Zhou et al. (1998), Rajewski et al. (1999) and Senneville et al. (2001). Today, DNA markers are more often used. They allow precise genome analysis, as they are mostly inherited in accordance with Mendelian genetics and are not subject to influences from the natural environment (Grzebelus, 1996).

In the literature there is very little information about the genetic diversity of tree species that grow in low numbers and play a small role in generating forest ecosystems (Senneville et al., 2001). Most authors do research on tree species that are widespread and numerous (Hamrick et al., 1992).

e-mail: rlzarek@cyf-kr.edu.pl

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TABLE 1. Location of the four studied natural populations of *Taxus baccata*

Population	Latitude	Longitude	Altitude m a.s.l.	No. of individuals in population	No. of sample trees
Nowa Wieś	49°31'40" N	21°42'10" E	430–470	41	30
Cisowa Góra	50°32'15" – 50°32'25" N	16°41'50" – 16°42'20" E	395–549	708	30
Zadni Gaj	49°00'44" N	18°00'45" E	440–519	43	30
Liswarta	50°46'59" N	18°46'01" E	250–255	576	30

Fig. 1. Map of the four natural populations of *Taxus baccata* used in this study.

RAPD markers have generally been used for *Taxus* sp. This method was used to study the variability of *Taxus baccata* in Italy (Paffetti et al., 1999) and Switzerland (Hilfiker et al., 2004). In North America, RAPD markers were used to create a gene map for *Taxus brevifolia* (Gocmen et al., 1996) and to research genetic diversity between individuals of *Taxus canadensis* (Corradini et al., 2002). Collins et al. (2003) looked for DNA markers to distinguish species (*Taxus baccata, Taxus canadensis, Taxus cuspidata*) and hybrids (*Taxus* × *media, Taxus* × *hunnawelliana*). In Asia, Shasany et al. (2000) and Mohapatra et al. (2009) investigated the genetic diversity of *Taxus wallichiana* populations originating from northern India. Shah et al. (2008) examined individuals of *Taxus fuana* from Pakistan.

Analysis of the genetic diversity of *Taxus* sp. with the use of molecular (isoenzyme and DNA) markers has not given an unequivocal answer as to whether *Taxus* is one widespread species with many subspecies or if the genus *Taxus* contains different species. Using isoenzymatic markers, Zhou et al. (1998) found that *Taxus cuspidata, Taxus yunnanensis* and *Taxus chinensis* are very similar, and that they are probably races of one species. On the other hand, Collins et al. (2003) report that it is very easy to distinguish *Taxus baccata, Taxus cuspidata* and *Taxus canadensis* by the RAPD method, making them separate species.

The genetic diversity of *Taxus baccata* among and within populations in Poland has not been investigated with DNA marker technique. In the Wierzchlas Nature Reserve, Lewandowski et al. (1995) used isoenzyme markers to examine the genetic structure of the yew population, and that is the only such study.

This work presents the results of research using RAPD markers to assess the genetic diversity of yew in southern Poland.

MATERIALS AND METHODS

PLANT MATERIAL

This study used four natural populations of *Taxus baccata* in southern Poland: two small ones (<100 individuals; Zadni Gaj, Nowa Wieś) and two large ones (>100 individuals; Cisowa Góra, Liswarta) (Fig. 1, Tab. 1). Plant material was collected in December. Needles from 30 individuals were collected from each population along a transect (large populations) or the first 30 individuals encountered (small populations). The needles were cut, frozen in liquid nitrogen and stored at -80°C until DNA extraction was done.

EXTRACTION OF DNA

Genomic DNA was extracted by the CTAB method (Carlson et al., 1991) with some modifications. Frozen needles (~200 mg) were ground in a mortar, the pulverized tissue was put into 1.5 ml sterile Eppendorf tubes and then was mixed with 1000 µl buffer containing 2% CTAB (Hexadecyltrimethylammonium bromide) (Sigma), 1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (Ethylene diamine tetraacetic acid), 1% PEG (Polyethylene glycol) and 0.2% β-mercaptoethanol. After shaking, the tubes were placed in a water bath at 65°C for two h. Then the solution was centrifuged for 10 min at 13,000 rpm (~17000 g). The supernatant was transferred to

TABLE 2. PCR reaction mixture components for each primer. Primers that did not produce any amplification products (not bolded) in the examined conditions were discarded

Nr	Primer	Sequence 5'→3'	Concentration of				
			magnesium ions [mM]	primer [μM]	dNTP [μM]	*Taq* polymerase [U]	DNA [ng]
1	**OPA 02**	**TGCCGAGCTG**	2.0	0.2	200	3	10
2	**OPA 04**	**AATCGGGCTG**	4.0	0.2	200	3	10
3	OPA 06	GGTCCCTGAC	–	–	–	–	–
4	OPA 07	GAAACGGGTG	–	–	–	–	–
5	OPA 08	GTGACGTAGG	–	–	–	–	–
6	**OPA 09**	**GGGTAACGCC**	4.0	0.2	200	3	10
7	**OPA 10**	**GTGATCGCAG**	4.0	0.2	200	3	10
8	**OPA 11**	**CAATCGCCGT**	3.0	0.2	200	3	10
9	**OPA 12**	**TCGGCGATAG**	4.0	0.2	200	3	10
10	OPA 13	CAGCACCCAC	–	–	–	–	–
11	**OPA 15**	**TTCCGAACCC**	4.0	0.2	200	3	10
12	OPA 16	AGCCAGCGAA	–	–	–	–	–
13	OPA 17	GACCGCTTGT	–	–	–	–	–
14	**OPA 18**	**AGGTGACCGT**	4.0	0.2	200	3	10
15	**OPA 19**	**CAAACGTCGG**	1.5	0.2	200	3	10

another tube, mixed with the same volume of a mixture of chloroform and isoamyl alcohol (24:1) and centrifuged for ten min at 13,000 rpm. The last step was then repeated. After collecting the aqueous phase, the same volume of cooled isopropanol was added in order to precipitate the DNA at -20°C for more than 10 h (overnight). After that time tubes were centrifuged for 10 min at 13,000 rpm. The resulting DNA pellet was rinsed with 200 μl 70% ethanol and dried at room temperature. The obtained nucleic acids were dissolved in 30 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with the addition of ribonuclease (concentration 10 $\mu g \cdot cm^{-3}$) and incubated for 24 h at 37°C. After that step the following modification was applied: 20 μl TE buffer with 50 $\mu g \cdot cm^{-3}$ k-proteinase (Fermentas) was added to each sample, which was then incubated for 24 h at 50°C. Then the obtained concentration of DNA was determined. For further research the DNA samples were stored at less than -20°C.

DNA AMPLIFICATION

Genomic DNA polymorphism was determined by the random amplified polymorphic DNA (RAPD) method (Williams et al., 1990; Tingey and del Tufo, 1993). Fifteen 10-nucleotide primers were used for DNA amplification, all of which had been randomly selected from primer group OPA01-OPA20 (Operon Technologies, Inc.). Amplification was conducted in sterile 0.2 ml Eppendorf tubes in 25 μl reaction mixture containing PCR buffer [750 mM Tris-HCl, pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% Tween 20]; $MgCl_2$ (concentration adjusted to the results in preliminary experiments); 200 μM each of dATP, dCTP, dGTP, dTTP; 0.2 μM primer; Taq DNA polymerase (Fermentas); and template DNA (concentration adjusted to the results in the preliminary experiments) (Tab. 2). The final volume was 25 μl reaction mixture. All analyses used reagents from Fermentas (www.abo.com.pl), and all primers were obtained from Operon Technologies Inc.

TABLE 3. Primers used in RAPD analysis, and number and size of generated bands

No.	Primer	Sequence 5'→3'	Total number of bands	Range of obtained DNA fragment lengths [bp]
1	OPA 02	TGCCGAGCTG	18	530–1930
2	OPA 04	AATCGGGCTG	16	450–1920
3	OPA 09	GGGTAACGCC	21	430–1920
4	OPA 10	GTGATCGCAG	21	440–1840
5	OPA 11	CAATCGCCGT	18	330–1660
6	OPA 12	TCGGCGATAG	22	530–1910
7	OPA 15	TTCCGAACCC	27	390–2000
8	OPA 18	AGGTGACCGT	24	330–1650
9	OPA 19	CAAACGTCGG	18	490–1920
Sum or range			185	330–2000

Amplification was conducted in a thermocycler (Biometra T3) for 3 min at 94°C (preliminary denaturation), 1 min at 94°C (denaturation), 1 min at 37°C (annealing) and 2 min at 72°C (elongation). Steps 2 to 4 were repeated 45 times. Final elongation was for 10 min at 72°C. Mixture without DNA was the negative control. After the PCR reaction, loading buffer [LB: 10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol, 60 mM EDTA] was added to each tube (5 µl LB + 25 µl PCR reaction products).

The amplification products were separated electrophoretically on 2% agarose gel (Prona) immersed in TBE buffer (Tris Borate EDTA buffer) (ICN Biomedicals, Inc.), and 0.2 $\mu g \cdot cm^{-3}$ ethidium bromide was added to the gel for visualization of bands. For electrophoresis, 20 µl reaction mixture with the loading buffer was used. Electrophoresis was conducted at 75 V for 3–4 h, and then photographs were taken under UV with a BioCapMw (Vilber Lourmat). Then the image was analyzed using Bio-1D++ (Vilber Lourmat).

The size of amplification products was determined with 3 µl DNA size marker (100 bp GeneRuler DNA Ladder Plus, Fermentas).

DATA ANALYSIS

RAPD phenotypes for each primer were scored as present (1) or absent (0) for each individual. The lengths of obtained fragments were determined with Bio-1D++ software. A band was assumed to be monomorphic if it appeared in all trees from the examined population.

Genetic variation within populations was estimated with Shannon's index (Lewontin, 1972) using POPGENE ver. 1.31 (Yeh et al., 1999). Shannon's index is suitable for analyzing RAPD data because of its insensitivity to the bias that can be introduced into data by the inability to detect heterozygous individuals (Dawson et al., 1995; Gillies et al., 1997; Parani and Parida, 1997; Gustafson et al., 1999; Maki and Horie, 1999; Oiki et al., 2001; Aga et al., 2003).

Analysis of molecular variance (AMOVA, Excoffier et al., 1992) was used to partition the total genotypic variance within and among populations. AMOVA was performed with GENALEX V6.1 (Peakall and Smouse, 2006). The molecular variance within each population was calculated as an indicator of intrapopulation genetic variation. Estimates of the partitioning of genetic variation among the four populations and among individuals within the populations were initially derived from a global analysis without considering differences within region. The significance of the variance components was tested by calculating their probabilities based on 999 random permutations.

The indicator of population genetic differentiation, pairwise Φ_{PT} from Φ-statistics (Excoffier et al., 1992; Peakall et al., 1995) of AMOVA was calculated, and correlations between pairwise Φ_{PT} values and geographic distance were tested with the Mantel test. The Euclidean metric was used as the basis for principal coordinate analysis (PCO) (Gower, 1966), from which graphical relationships between individuals were derived.

RESULTS

The nine selected RAPD primers allowed us to obtain 185 DNA fragments, an average 21 per primer (Tab. 3). OPA 04 primer gave the fewest

TABLE 4. Specific DNA fragments (monomorphic for population or observed only in a given reserve)

Primer	Length of specific DNA fragments [bp]	Reserve			
		Nowa Wieś	Cisowa Góra	Zadni Gaj	Liswa rta
OPA 02	1500	P	P	M	M
	1430	P	M	M	M
	1050	P	P	P	M
	970	P	P	P	M
	880	P	P	M	P
	790	M	P	P	M
	660	A	P	A	A
	530	A	A	P	A
	720	P	P	M	M
OPA 04	1920	A	A	A	P
	1320	P	P	P	M
	1150	M	M	M	M
	890	A	A	A	P
	840	M	M	M	M
	610	P	P	M	P
	540	P	P	M	M
OPA 09	1730	P	P	P	M
	1500	P	P	M	M
	1200	P	P	M	P
	800	P	M	M	M
	580	A	A	P	A
	540	P	A	A	A
	430	A	P	A	A
OPA 10	1410	P	P	P	M
	1310	P	P	P	M
	1180	M	M	M	M
	1000	M	P	P	M
	960	M	M	P	M
	930	P	A	A	A
	790	P	A	A	A
	760	M	P	P	M
OPA 11	330	M	P	M	M
OPA 12	1110	P	P	M	M
	870	A	A	P	A
OPA 15	1550	P	P	P	M
OPA 18	1620	M	M	M	M
	1100	P	P	M	M
	900	M	P	M	M
	460	M	P	M	P
	330	M	P	M	A
OPA 19	1340	P	P	P	M
	650	P	P	P	M
	570	P	A	M	A

M – monomorphic fragments; P – polymorphic fragments; A – absent DNA fragments.

bands (16 fragments) and OPA 15 the most (27 fragments). Prior optimization of the magnesium ion concentration allowed a sufficient number of bands to be obtained for each primer used.

TABLE 5. Number of polymorphic bands, Shannon's index and standard deviation within each of four *Taxus baccata* populations

Population	Number of polymorphic bands	Percentage of polymorphic bands	Shannon's index*
Nowa Wieś	143	77.30%	0.3417a
Cisowa Góra	143	77.30%	0.3519a
Zadni Gaj	139	75.14%	0.3326a
Liswarta	90	48.65%	0.2161b
Mean	128.75	69.59%	0.3106

* Values with the same letter in a column do not differ significantly at $\alpha = 0.05$

Four DNA fragments (1150 bp and 840 bp, OPA 04; 1180 bp, OPA 10; 1620 bp, OPA 18) were monomorphic for all investigated populations (Tab. 4). Because I did not obtain data for other populations and species of *Taxus*, I could not be certain whether these fragments were characteristic of the entire *Taxus baccata* species or only the investigated populations. The primers used did not provide single markers to distinguish one population from another. Thus, classification of individuals to a specific population was done by band pattern rather than single markers. The number of polymorphic bands was 128.75 on average, ranging from 90 (Liswarta) to 143 (Nowa Wieś and Cisowa Góra). The average polymorphism level was 69.6%, ranging from 48.6% to 77.3% depending on the individual's origin. Shannon's indices ranged from 0.216 (Liswarta) to 0.352 (Cisowa Góra), averaging 0.311. Two groups of populations were distinguished by ANOVA: those growing in mountains (Nowa Wieś, Cisowa Góra and Zadni Gaj), with a higher level of internal diversity, and those growing in the lowlands (Liswarta), with a lower level of internal diversity (Tab. 5).

AMOVA revealed 26% genetic variation between populations and 74% within populations. All AMOVA variation was highly significant ($p < 0.001$) (Tab. 6).

Pairwise Φ_{PT} values and geographic distances between the four populations are shown in Table 7. The pairwise Φ_{PT} values ranged from 0.204 (Cisowa Góra and Zadni Gaj) to 0.324 (Nowa Wieś and Liswarta), and each of the pairwise Φ_{PT} values was significant according to tests based on 999 random permutations ($p < 0.001$). No significant correlation between pairwise Φ_{PT} and geographic distance was found by the Mantel test, suggesting that geographic distance had no effect on genetic variation. The "isolation by distance" model was not supported. The

TABLE 6. Summary of AMOVA results

Source	df	SS	MS	Est. Var.	%	p
Among populations	3	654.858	218.286	6.643	26%	<0.001
Within populations	116	2201.833	18.981	18.981	74%	<0.001
Total	119	2856.692		25.625	100%	

ss – sum of squares; ms – mean squares; est. var. – estimated variability; % – proportion of genetic variability; p – significance level.

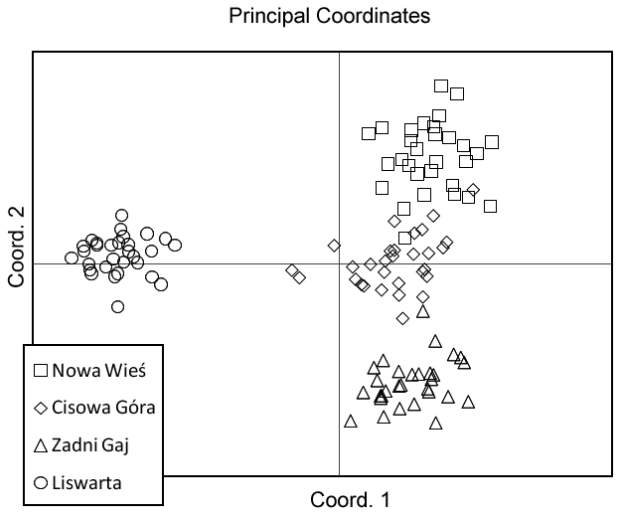

Fig. 2. Plot of the first two principal coordinates for each individual of four natural *Taxus baccata* populations. Projection into first and second coordinates; 31.04% and 20.97%. of variability is explained by the first two axes.

first three principal coordinates derived from PCO based on the Euclidean metric for the sampled individuals of the four populations described 31.04%, 20.97% and 20.47% of total variance. The first two coordinates are plotted in Figure 2; the four populations were separated into two clusters. One cluster included the three populations from mountains (Nowa Wieś, Cisowa Góra and Zadni Gaj) and the other cluster grouped the remaining individuals from the Liswarta (lowland) population.

DISCUSSION

In this work, RAPD analyses provided insight into the genetic diversity, genetic structure and distribution of the four native populations of *Taxus baccata* in southern Poland. It produced enough polymorphic markers for estimation of population genetic parameters, meeting the critical number of dominant markers suggested for reliable estimation of population genetic parameters (Mohapatra et al., 2009). The nine primers used gave 185 DNA fragments, an average 20 per primer (Tab. 2). Corradini et al. (2002) had similar results in work on *Taxus canadensis*. The seven primers they used gave 125 DNA fragments, an average 18 per primer (range 10–24). In a study of genetic diversity in *Taxus wallichiana*, Shasany et al. (2000) obtained only 106 fragments from 15 primers (average 7 PCR products per primer). RAPD produced 69.5% polymorphic bands (48.6% to 77.3% depending on the individual's origins). According to Shasany et al. (2000) and Mohapatra et al. (2009) populations growing in India had lower polymorphism (45% and 43%, respectively), but this may have been due to small sample size. As long-lived dioecious gymnosperms, theoretically the level of genetic variation in *Taxus* should be relatively high within populations and relatively low between populations (Hamric et al. 1992). Here the Shannon index ranged from 0.216 (Liswarta) to 0.352 (Cisowa Góra), averaging 0.311. For comparison, the Shannon's index ranges and mean values were 0.349–0.612 and 0.547 for 12 populations of the South American conifer *Fitzroya cupressoides* (Allnut et al., 1999), 0.592–0.733 and 0.651 for eight populations of *Populus tremuloides* (Yeh et al., 1995), 0.301–0.367 and 0.332 for six populations of the endangered tropical tree *Plathymenia reticulate* (Lacerda et al., 2001), and 0.360–0.460 and 0.405 for four populations of *Betula maximowicziana*, a long-lived pioneer tree species (Tsuda et al., 2004). Nybom and Bartish (2000) determined the average values of Shannon's index for other gymnosperms (0.386) and other long-lived perennial plants (0.242). The within-population genetic variation of *Taxus baccata* in southern Poland appears high compared to those results. Corradini et al. (2002) reported similar levels of within-population genetic variation for *Taxus canadensis* using RAPD (0.360) and AFLP (0.300). Low population-level genetic variation has been found in other *Taxus* species: *Taxus wallichiana* (0.230 to 0.020) (Shasany et al., 2000), *Taxus canadensis* (Senneville et al., 2001), *Taxus brevifolia* (El-Kassaby and Yanchuk, 1994) and *Taxus fuana* (0.179) (Shah et al., 2008). The different values for *Taxus baccata* may be attributable in part to the use of different RAPD primers rather than to ecology or to a difference in their reproductive biology, as *Taxus* species are all wind-pollinated and bird or vertebrate dispersed. *Taxaceae* are a relict family which according to the fossil evidence originated in the lower or middle Jurassic (Florin, 1958). In gen-

TABLE 7. Pairwise Φ_{PT} (bellow diagonal) and geographic distance in km (above diagonal) between examined populations of *Taxus baccata*.

Population	Nowa Wieś	Cisowa Góra	Zadni Gaj	Liswarta
Nowa Wieś	–	368	209	242
Cisowa Góra	0.206	–	169	148
Zadni Gaj	0.211	0.204	–	111
Liswarta	0.324	0.304	0.313	–

Each pairwise Φ_{PT} value was significant ($P < 0.001$).

eral, geographically widespread species tend to possess higher genetic polymorphism within populations than species with a restricted distribution (Shah et al., 2008).

The genetic structure of plant populations reflects the interactions of various evolutionary processes including the long-term evolutionary history, shifts in distribution, habitat fragmentation and population isolation, mutation, genetic drift, mating systems, gene flow and selection (Schaal et al., 1998). The genetic differentiation values detected by AMOVA among populations of *Taxus baccata* growing in southern Poland (26% of the genetic variation between populations and 74% within populations – Tab. 6, $\Phi_{PT} = 0.26$) were similar to the average for outcrossing plant species ($\Phi_{PT} = 0.27$) and for gymnosperms ($\Phi_{PT} = 0.24$) (Nybom and Bartish, 2000). Within-population variation accounted for most of the genetic variation of *Taxus baccata*. That is typical for outcrossing, wind-pollinated and long-lived woody zoochores (Shah et al., 2008). For example, total within-population genetic variation was 84% for four *Abies* (three *Abies alba* and one *A. nebrodensis*) populations (Vicario et al., 1995), 97.4% for eight *Populus tremuloides* populations (Yeh et al., 1995), 63% for six *Pseudotsuga menziesii* populations (Aagaard et al., 1998), 87.7% for six *Plathymenia reticulate* populations (Lacerda et al., 2001), 85.6% for 12 *Fitzroya cupressoides* populations (Allnut et al., 1999) and 80.2% for 37 *Eucalyptus globulus* populations (Nesbitt et al., 1995). As in other studies of long-lived woody perennials and wind-pollinated species, my AMOVA results show higher genetic variation within populations than between them (Hamrick and Godt 1990; Franjić 1996; Baliuckas et al. 1999; Rehfeldt 1999; Kovaèić and Šimić 2001; Bruschi et al., 2003; Hilifiker et al., 2004).

Taxus species differ in their levels of genetic diversity. According to Senneville et al. (2001) the lowest diversity is in *Taxus canadensis*; it is slightly higher for *Taxus brevifolia* (El-Kassaby and Yanchuk 1994, Wheeler et al., 1995), and highest for *Taxus baccata* (Lewandowski et al., 1995). My results confirm the high within-population diversity of *Taxus baccata* growing in southern Poland, in accord with Lewandowski et al.'s (1995) isoenzyme marker results from the Wierzchlas Reserve in northern Poland.

The yew seems more variable than the other conifers (Ledig 1986). Most likely this is because *Taxus* was once a widespread species with a compact range, whereas now there are only small scattered populations. This is supported by paleontological data (Środoń 1975). Other factors also affect the high genetic diversity of *Taxus baccata*: dioecism, anemophily and longevity. High adaptability and a wide geographic range also are associated with high genetic diversity (Hamrick, 1989; Bush and Smouse, 1992). From the conservation perspective, high variability and high level of gene flow between populations is encouraging. The wind-borne pollen of *T. baccata* can be dispersed over large distances. Dispersal of seeds by birds or small mammals in this species may contribute to long-distance gene flow, for example when migrating birds feed on the seeds and excrete them later on (Hilfiker et al., 2004).

This study used RAPD technique to produce basic genetic information about natural populations of *Taxus baccata* in southern Poland. There is still no set of genetic diversity data obtained by one method and covering all of Poland for *Taxus baccata*. To obtain detailed genetic information that will facilitate conservation and management of forest ecosystems containing *Taxus baccata*, future studies should be wide-ranging and should apply fine-scale analysis using molecular markers.

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