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Genetic diversity of lowland and montane populations of *Polygonatum verticillatum* (L.) All. determined on the basis of isozymatic analysis

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Abstract: This study aimed to determine the genetic diversity of *Polygonatum verticillatum* (L.) All., and to estimate the degree of genetic similarity between 18 Polish populations of this species: 9 from the lowlands in the north and 9 from the mountains in the south of Poland. Five enzymatic systems (AAT, EST, GDH, LAP and MDH) revealed 13 loci. Polymorphism was observed at the *Aat*3 locus and all esterase loci. An isozymatic analysis did not show any significant differentiation between lowland and montane populations, and genetic similarity between the two groups was high ($I = 0.95$). The results suggest that in the past this species had a continuous geographic range in Poland or that the populations examined originated from the same geographic refuge.

Key words: *Polygonatum verticillatum*, Convallariaceae, montane species, genetic diversity, isozyme electrophoresis

1. Introduction

The current distribution ranges of European plant species are a consequence of numerous climatic and habitat changes that took place during and after the Pleistocene glaciations. Plants survived in glacial refuges on the Iberian and Apennine Peninsulas, and in the Balkans. After glaciations they spread widely across postglacial areas (Hewitt 1999). These migrations not only affected the current ranges of taxa, but also their intraspecific genetic variation. The species that originated from large populations remained genetically diverse. On the other hand, migrations from small, isolated populations contributed to a decrease in genetic variation (Hewitt 1989, 1999).

A large group of European species have had disjunctive ranges since the glacial period. This fact may have serious consequences, provided that the geographic distance is big enough to hinder or prevent gene flow between populations. In such a situation there appear differences between them, usually concerning the frequency of alleles and genotypes, as well as the presence of unique alleles.

One of the plants that have a disjunctive range in Poland is whorled Solomon's seal *Polygonatum verticillatum* (L.) All., which belongs to the family Convallariaceae. This species is the only European representative of the section *Verticillata* Baker. This is an arctic-alpine montane species whose global range comprises the mountain areas of Europe and Asia (the Cantabrian Mts., Pyrenees, Alps, Apennines, Dinaric Mts., Sudeten, Carpathians, Pontic Mts., Caucasus and Himalayas). This species is also very frequent in the Arctic zone in Europe (Meusel & Jäger 1992). In Poland whorled Solomon's seal is common in the Sudeten and Carpathian Mts. It is also known from lowland localities, situated in the Sudetian-Carpathian Foreland and in northern Poland (Szafer 1930; Czubiński 1950; Polakowski 1963; Jakubowska-Gabara & Jost-Jakubowska 1978; Zając 1996). Due to the fact that the distribution ranges of some species known from the Kolbusz Upland and those recorded from low-mountain regions overlap, whorled Solomon's seal is considered an incomplete relict species (Dubiel *et al*. 1983). Literature data show that *P. verticillatum* can be found across a very wide spectrum of habitat conditions. In the mountains it is common in communities of tall grasses and herbaceous plants, with an abundant supply of well-oxygenated water, on steep slopes of valleys and chutes in hillside forests dominated by sycamores, at the bottoms of periodically

dry streams, and along brooks. It has been also reported from localities in spruce- and beech-dominated communities in high-mountain regions. In the lowlands it usually occurs in forest communities of the alliances *Carpinion* and *Fagion* (Matuszkiewicz 2001; Pawłowski 1977).

The number of chromosomes in *P. verticillatum* varies, but in the case of the European populations of this species 2n=28 (Skalińska & Pogan 1971). Individuals whose number of chromosomes is 2n=56+1B (Therman 1953; Polatschek 1966), 2n=30 (Pandita & Mehra 1982), 2n=60 (Therman 1953) and 2n=90 (Suomalainen 1947; Therman 1953) have been reported from Asia. Tamura (1997), who conducted phylogenetic research on 23 species of the genus *Polygonatum* by using chloroplast DNA, found that *P. verticillatum* is closely related to *P. prattii* Baker – an endemic species known only from localities in China.

Whorled Solomon's seal shows iterative growth. It produces sympodial, creeping rhizomes that play a major role in reproduction. This is an autogamous plant and flowering extends from the end of May to the beginning of June.

Basing on an analysis of its distribution range in Poland, many authors concluded that the populations from northern Poland are genetically related to those from the Scandinavian distribution center, and that they differ from montane populations (Szafer 1930; Czubiński 1950; Polakowski 1963). However, there are no sufficient published genetic data that would confirm this hypothesis. The geographic distance between the lowland and montane populations could result in considerable genetic differences between them. Also the fact that these plants may come from distant refuges could have a significant effect on allele frequency.

The aim of the present work was to study the genetic differentiation between the lowland and montane populations of this species.

2. Material and methods

2.1. Plant material

The study was performed on 18 population samples from Poland, including 9 from the mountains and 9 from the northern part of the country (Fig. 1). Samples were collected in the years 2002-2003. Sample size varied from 5 to 15, depending on population size (Table 1). The experimental materials comprised rhizomes that were grown for 2 years on a trial plot. This permitted their collection at a fixed time, and enabled to exclude habitat-related and seasonal variability in the expression of tested enzymes.

Fig. 1. Distribution of analyzed *Polygonatum verticillatum* populations

2.2. Allozyme analysis

The analysis was carried out for 180 individuals by using horizontal starch electrophoresis. The following 5 enzyme systems were assayed: aspartate aminotransferase

Table 1. Characteristics of analyzed montane (M) and lowland (L) populations

Population	Location	Habitat type	Population size (N)	Sample size (n)
M-Opa	Opawskie Mts.	*Dentario enneaphylli-Fagetum*	55	9
M-Tza	Western Tatra Mts.	*Betulo-Adenostyletea*	70	8
M-Mly	Młynowiec	*Carici remotae-Fraxinetum*	400	14
M-Gbi	Polana Bieniowe	*Luzulo luzuloidis-Fagetum*	64	9
M-Twy	High Tatra Mts.	*Polysticho-Piceetum*	79	7
M-Bia	Bialskie Mts.	*Luzulo luzuloidis-Fagetum*	60	10
M-Krk	Karkonosze Mts.	*Calamagrostio villosae-Piceetum*	45	7
M-Zlo	Złote Mts.	*Luzulo luzuloidis-Fagetum*	450	10
M-Gka	Tributary of Kamieniec	*Luzulo luzuloidis-Fagetum*	40	7
L-Rom	Romnicka Forest	*Fraxino-Alnetum*	500	10
L-Kud	Kudypy Forest District	*Tilio-Carpinetum*	650	15
L-Sta	Stańczyki	*Tilio-Carpinetum*	202	9
L-Wpk	Welski Landscape Park	*Tilio-Carpinetum*	110	13
L-Mpk	Masurian Landscape Park	*Tilio-Carpinetum*	110	15
L-Suw	Suwałki Landscape Park	*Fraxino-Alnetum*	50	5
L-Str	Strzałowo	*Tilio-Carpinetum*	80	5
L-Ipk	Iława Landscape Park	*Tilio-Carpinetum*	71	15
L-Red	Reda	*Fraxino-Alnetum*	28	5

(AAT), esterases (EST), glutamic acid dehydrogenase (GDH), leucyloaminopeptidase (LAP), and malate dehydrogenase (MDH). Extractions were performed by using glass pestles on cooled plastic plates and 3 drops of 2% B-mercaptoethanol added per 4-cm^2 leaf sample. Extracts were absorbed onto paper wicks and loaded onto 11% starch gel. Electrophoresis was performed in 0.1 M lithium-borate buffer at pH 8.3 (Zieliński 1987). Gels were run for 6 h in 4^{O}C at 300V and 60 mA. The staining of the enzyme systems was carried out following Soltis & Soltis (1989) with modifications.

2.3. Data analysis

The following measures of diversity were calculated by using POPGENE V. 1.31 (Yeh & Boule 1999): (*i*) percentage of polymorphic loci (*P*), (*ii*) mean number of alleles per locus (*A*), (*iii*) total genetic diversity (H_T), (*iv*) intrapopulation diversity (H_S), (*v*) interpopulation diversity (D_{ST}), and (*vi*) proportion of total genetic diversity found between populations ($G_{ST} = D_{ST}/H_T$). A locus was considered polymorphic if the frequency of its most common allele did not exceed 0.95. Genetic similarities between populations calculated according to Nei's (1972) genetic identity, were summarized in an UPGMA phenogram. The correlations and significance tests were performed in the computer program Statistica 6.0 (StatSoft 2003).

3. Results

3.1. Genetic variation within and among populations

An analysis of 5 enzyme systems, revealed 13 loci. Most of them were revealed by esterase (5 loci), and only 1 locus by glutamic acid dehydrogenase (Table 2).

Table 2. Allele frequencies at 6 polymorphic loci of montane and lowland populations *Polygonatum verticillatum*

Locus	Alleles	Montane populations	Lowland populations
Aat3	12	0.589	0.352
	9	0.411	0.648
Est1	52	0.614	0.186
	49	0.429	0.373
	null	0.013*	0.384*
Est2	47	0.386*	0.785*
	45	0.170	0.095
	null	0.519*	0.044*
Est3	34	0.349	0.352
	null	0.658	0.684
Est4	27	0.418	0.462
	null	0.582	0.539
Est5	24	0.139	0.363
	null	0.861	0.637

Among the 13 loci analyzed, all esterase loci and the *Aat*3 locus showed polymorphism, and the others were monomorphic. The mean percentage of polymorphic loci per population was $P = 25.2\%$. However, particular populations differed considerably in the percentage of polymorphic loci. These differences were correlated ($r = 0.58$) with population size and the type of habitat occupied by populations (0.00-46.15%, Table 3).

Table 3. Mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), intrapopulation diversity (H_S unbiased estimate; Nei 1978)

Population	*A*	*P*	H_S
M-Opa	1.2	24	0.08
M-Tza	1.5	46	0.22
M-Mly	1.3	38	0.14
M-Gbi	1.3	31	0.15
M-Twy	1.2	15	0.09
M-Bia	1.1	8	0.04
M-Krk	1.5	15	0.05
M-Zlo	1.3	31	0.15
M-Gka	1.5	15	0.05
L-Rom	1.3	31	0.11
L-Kud	1.5	46	0.19
L-Sta	1.2	23	0.1
L-Wpk	1.3	31	0.14
L-Mpk	1.4	39	0.16
L-Suw	1.0	0	0
L-Str	1.2	15	0.68
L-Ipk	1.2	15	0.02
L-Red	1.5	15	0.05
Mean	1.23	25	0.09

The total number of alleles was 21 and the mean number of alleles per locus was 1.23 and ranged from 1.00 to 1.50 (Table 3). The mean total genetic diversity was $H_T = 0.232$. The mean intrapopulation diversity (H_S) was 0.09. The population from the Western Tatra Mts. and the population located in the Forest District Kudypy showed the highest intrapopulation diversity: $H_S = 0.22$ and $H_S = 0.19$, respectively (Table 3). No polymorphism was observed in the population from the Suwałki Landscape Park. The populations differed also in the percentage of polymorphic loci and allele frequency, which was reflected by a slightly higher value of interpopulation diversity ($D_{ST} = 0.15$) than intrapopulation diversity ($H_S = 0.09$). It was found that interpopulation diversity accounts for 55% ($G_{ST} = 0.55$) of total diversity. The greatest differences were observed between the population from the Opawskie Mts. and the population from the Iława Landscape Park ($I = 0.67$). The highest degree of similarity was found between the population from Reda and the population from the Gorce National Park ($I = 0.99$). Population grouping by UPGMA clustering revealed 2 population groups (Fig. 2). The low genetic similarity was confirmed by great genetic distances, which ranged between 0.01 and 0.39.

3.2. Geographic differentiation

The lowland and montane populations showed various allele frequencies. The *Est*2-52 alleles and the null allele at the *Est*2 locus, characterized by much higher frequency in the montane populations, were the

most effective in terms of differentiating between the lowland and montane populations (Table 2). However, we did not find any allele that would be present in all montane populations and absent in the lowland ones. There were also differences in H_T values calculated for particular loci. In the montane populations the highest H_T value was recorded at the *Est*2 locus (0.576), and the lowest at the *Est*5 locus (0.241), whereas in the lowland populations at *Est*1 (0.637) and *Est*2 (0.353), respectively. Similar values of this coefficient were obtained for the lowland and montane populations (0.22 and 0.21, respectively). The montane populations showed a minimally higher intraspecific diversity (H_S = 0.1), compared with the lowland populations (H_S = 0.09). Differences in the percentage of polymorphic loci were also minimal. The mean percentage of polymorphic loci was 24.7% in the montane populations and 23.9% in the lowland ones. The genetic similarity between the lowland and montane populations was high (I = 0.95).

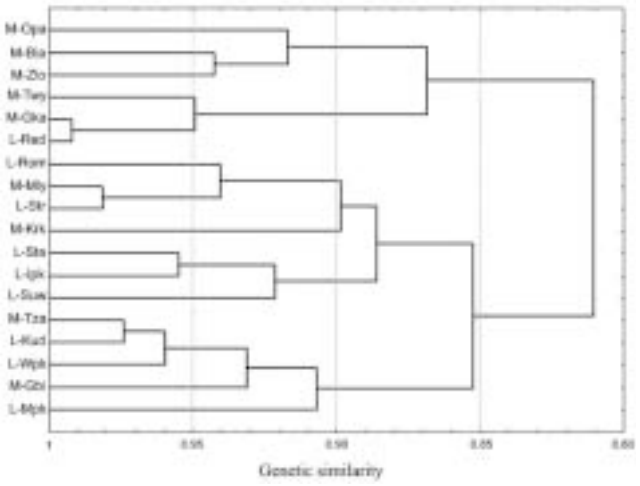

Fig. 2. UPGMA dendrogram of 20 populations of *Polygonatum verticillatum*, based on Nei's (1972) genetic identity

4. Discussion

The parameters of genetic variation expressed as means of all populations, were as follows: P = 25.2%, A = 1.23 and H_S = 0.09 (Table 3). These values are somewhat lower than those summarized by Hamrick & Godt (1990) for vascular plants. Those authors studied about 500 species of vascular plants, and reported the following mean parameters of genetic variation: P = 34.2% (range 29.0-57.7%), A = 1.53 (range 1.44-1.93), H_S = 0.113 (range 0.096-0.160). The fact that the values of these parameters were lower in whorled Solomon's seal may result from vegetative reproduction of this species.

Researchers differ in their opinions on genetic variation in clonal plants, as compared with generatively reproducing species. Some authors (e.g. Williams 1975) believe that the populations of plants that reproduce vegetatively should have several local genotypes, whereas others share the view that asexual populations may be equally polymorphic as the populations that reproduce sexually (e.g. Price & Waser 1982).

The populations analyzed in this study differed considerably in the degree of genetic variation. The greatest diversity was recorded in the populations from the Western Tatra Mts. and from Kudypy in the lowlands. They had the highest numbers of various genotypes, the highest percentage of polymorphic loci (P = 46%), the highest number of alleles per locus (A = 1.5) and the highest intrapopulation diversity (Western Tatras 0.22, Kudypy 0.19). Values of these parameters were much lower in the populations from the Opawskie Mts., Bialskie Mts. and the Iława Landscape Park. The population from the Suwałki Landscape Park was genetically uniform and composed of a single genotype only. These differences were found to be correlated with habitat type, since the less numerous populations from shaded forest communities were less variable than the populations from open, sunny habitats.

In contrast to sexually reproducing plants, in clonal species interpopulation diversity accounts for a greater part of total diversity than intrapopulation diversity does. The differences in the percentage of polymorphic loci and alleles increased the coefficient of interpopulation diversity (D_{ST}=0.13), and decreased the coefficient of genetic similarity between the 18 populations examined in the study. This indicates limited gene flow between the populations of whorled Solomon's seal, which is the main factor increasing genetic similarity.

The presence of alleles representing various frequency classes in the populations also increased the value of G_{ST}, which was equal to 0.55 in *P. verticillatum*. An analysis of G_{ST} in various vascular plant groups indicates correlations between the value of this coefficient and the survival strategies of particular species (Hamrick & Godt 1990). The lowest value of G_{ST} was recorded in species characterized by a long life cycle, cross-fertilization, and wind pollination, and the highest in autogamous species with a short life cycle. Therefore, it is not surprising that the value of this coefficient was high in whorled Solomon's seal, which is a self-pollinated and clonal plant.

Our results do not confirm the hypothesis proposed by Czubiński (1950) and Polakowski (1963), who concluded that the lowland populations of this species are genetically related to those from the Scandinavian distribution center and differ from the montane ones. No significant differences in H_S, H_T, P, and A values were found between the populations representing these two geographic regions. The coefficient of genetic similarity between them was high (I = 0.95). The number of alleles per locus (A) and the mean percentage of

polymorphic loci (*P*) were at similar levels in the lowland and montane populations (1.45 vs. 1.50 and 24.7% vs. 23.9%, respectively). The mean values of H_T were also similar in the lowland and montane populations (0.21 and 0.22, respectively). The level of intrapopulation diversity (H_S) was 0.09 in the lowland populations and 0.1 in the montane ones. The differences found in allele frequency (Table 2) may indicate the onset of a microevolutionary process. The great geographic distance between the lowland and montane populations of whorled Solomon's seal, preventing gene flow between them, as well as different environmental conditions of these populations, could contribute to this process.

Our results suggest that in the past *Polygonatum verticillatum* had a continuous geographic range in Poland, or that the populations examined originated from the same geographic refuge.

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