Genetic structure of *Artemisia pancicii* populations inferred from AFLP and cpDNA data

Genetická struktura populací *Artemisia pancicii* odvozená z AFLP a cpDNA dat

Miloslav K i t n e r[1], Ľuboš M a j e s k ý[1], Lenka G i l l o v á[2], Tomáš V y m y s l i c k ý[3] & Matthias N a g l e r[4,5]

[1]*Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic, e-mail: miloslav.kitner@upol.cz, lubos.majesky @gmail.com;* [2]*Agency for Nature Conservation and Landscape Protection of the Czech Republic, Olomouc Regional Office, Lafayettova 13, 779 00 Olomouc, Czech Republic, e-mail: lenka.gillova@nature.cz,* [3]*Agricultural Research, Ltd., Zahradní 1, 664 41 Troubsko, Czech Republic, e-mail: vymyslicky@vupt.cz;* [4]*Department of Conservation Biology, Vegetation and Landscape Ecology, Faculty of Life Sciences, Rennweg 14, A-1030 Vienna, Austria;* [5]*Department of Molecular Systems Biology, Faculty of Life Sciences, Althanstrasse 14, A-1090 Vienna, Austria, e-mail: matthias.nagler@univie.ac.at*

Kitner M., Majeský Ľ., Gillová L., Vymyslický T. & Nagler M. (2012): Genetic structure of *Artemisia pancicii* populations inferred from AFLP and cpDNA data. – Preslia 84: 97–120.

Genetic variability within and among fragmented populations of *Artemisia pancicii* was investigated in order to obtain a general understanding of the genetic structure related to the successful protection of this highly endangered species. Genetic variation within and among 15 populations of *A. pancicii* in Central Europe was analysed using amplified fragment length polymorphism (AFLP) and sequencing of two chloroplast DNA regions. The resulting polymorphism of AFLP loci was interpreted using basic population genetic indices and statistical visualisation. The total genetic variability within the populations was high (H_t = 0.248) and a highly differentiated population pattern (F_{st} = 0.241) was revealed. An analysis of molecular variance (AMOVA) revealed high variation among the populations (82%). There was no significant correlation between the genetic and geographic distance matrices. This indicates that population relatedness is not reflected in their geography. This was also confirmed by cpDNA sequencing. Highly restricted gene flow among the populations and genetic drift has resulted in reduced genetic variability in the smaller and highly differentiated *A. pancicii* populations, and very probably implies the presence of self-incompatibility and prevalence of clonal reproduction. The conservation of genetic variability in *A. pancicii* requires the persistence of large and also of small populations (because of population differentiation). The most important factor for the preservation of this species in the localities studied is the application of appropriate conservation management (such as mowing, grazing or fire management).

K e y w o r d s: *Artemisia pancicii*, AFLP, conservation, chloroplast DNA, endangered species, genetic variability

Introduction

The genus *Artemisia* L. (*Asteraceae*, *Anthemideae*) is the largest of the *Anthemideae* tribe and one of the largest in the *Asteraceae* family. It comprises from 200 to more than 500 taxa at the specific or subspecific level, depending on the taxonomic concepts adopted (see Vallès & McArthur 2001 and references thereinafter). *Artemisia* is a widely distributed genus in the northern hemisphere, with its centres of diversity in temperate and cold

temperate regions (Bremer 1994), and is rather scarce in the southern hemisphere. It is more frequent in Eurasia than North America. The region of wider Central Asia represents its main centre of speciation and diversification (McArthur & Plummer 1978, Vallès & McArthur 2001). The vertical distribution of the *Artemisia* taxa covers altitudes from sea level to high mountains, and they frequently colonize semiarid environments. Some *Artemisia* species occur in isolation, but more commonly they form extensive, landscape-dominant populations, especially in the arid regions of the world. Most of the species in the genus are perennial; only around fifteen are annual or biennial. Polyploidy is a frequent phenomenon in this genus with two basic chromosome numbers (x = 8 and x = 9), but dysploidy is also recorded (Hayat et al. 2009).

Artemisia pancicii Ronniger ex Danihelka et Marhold is a perennial herbaceous hemicryptophyte with a well-developed rhizome system that spreads and develops into a large cluster of above-ground ramets, which are mainly sterile and 5–10 cm tall, with 3–5 deeply lobed gold-tomentose (in the Czech Republic and Austria) or grey-white (in Serbia) hairy leaves. Flowering stems occur infrequently, and are (20–) 30–90 (–95) cm tall, with a leafy panicle with small, nearly globular flowerheads with anemochoric dispersal mechanisms. Achenes are ellipsoidal and only 1 mm long (Grulich 2004). It rarely grows in semi-dry grasslands or steppes, in vegetation of the alliances *Bromion erecti* or *Festucion valesiacae* over *Geranion sanguinei* to *Quercion pubescentis-sessiliflorae* and *Prunion spinosae* (Nagler 2010). This species prefers dry, shallow and carbonate-rich soil (Wendelberger 1959). Being a rhizomatous plant, *A. pancicii* grows in small or large patches of one or several clones.

Artemisia pancicii is a member of the subsect. *Laciniatae* (Kitam.) Korobkov (Korobkov 1992, Meusel & Jäger 1992). This group of plants is widely distributed from the Far East and Manchuria to Central Europe, with the centre of diversity in the mountain regions of south Siberia and Mongolia (Danihelka 1995). In Europe the following species of this subsection are found: *A. laciniata*, *A. atrata*, *A. oelandica*, *A. pancicii* and *A. insipida* (Tutin et al. 1976), which is considered to be the closest relative of *A. pancicii* (Ehrendorfer 1964).

Artemisia pancicii is a hexaploid species 2n = 54 (Ehrendorfer 1964, Rotreklová et al. 2004), supposedly a result of the hybridization of diploid *A. laciniata* and tetraploid *A. armeniaca*, as stated in Ehrendorfer (1964). However, the ploidy of *A. armeniaca* was inferred on the basis of pollen measurements of various species of various ploidy levels, and stated as "with high probability tetraploid" (Ehrendorfer 1964). To date, the only published chromosome counts (known to the authors) for *A. armeniaca* are 2n = 18 (diploid) (Torrell et al. 2001) and 2n = 54 (hexaploid) (Probatova et al. 2010). Thus, it seems that *A. pancicii* could be a hybrid between 2x *A. laciniata* and 6x *A. armeniaca*. However, further study is required to confirm this.

Artemisia pancicii is considered to be a late-glacial relict from the end of the Glacial and the beginning of the Preboreal, when changing climate allowed the spread of wooded-steppe elements into Central Europe, of which the members of the section *Laciniatae* are typical (Wendelberger 1960, Ehrendorfer 1964, Jäger 1987). It is considered to be one of the rarest native plants of Europe. As such it is a Pannonian endemic occurring only at 10 isolated localities in the Czech Republic, Austria and Serbia. There are three localities in southern Moravia in the Czech Republic (Danihelka 1995, Holub & Grulich 1999), six localities harbouring 13 populations in Niederösterreich (Lower Austria) and Burgenland – Federal Provinces in Austria (Nagler 2010) – and one locality in the surroundings of Deliblato in Vojvodina, Serbia (Boža 1999). All the populations of this rare wormwood

are spatially small, and altogether consist only of thousands of ramets. The rarity and high risk of extinction of this species is the reason why it is included in Annexes II and IV of the Habitats Directive (92/43/EEC). According to Habitats Directive Article 17 of the European Environment Agency, the conservation status is "unfavourable bad" as a result of the decrease in the extent of the already small habitat area and resultant small population size, low genetic variability and poor fertility (European Topic Centre on Biological Diversity 2009). Furthermore, this species is associated with habitat 6240 (Sub-Pannonic steppic grasslands) of Annex I in the Habitats Directive.

According to the concept of rarity (Rabinowitz 1981) *A. pancicii* belongs to the group of species that are naturally rare, with a historically narrow geographical range and with narrow habitat specialization. This species has become extinct at three localities in the Czech Republic (Danihelka 1995) and several in Austria; every locality (historical and recent) is isolated. *Artemisia pancicii* has survived in isolated populations for a long time and so, like many other naturally rare species, may be relatively more tolerant to negative processes affecting small populations (e.g. adapted for inbreeding or low availability pollen) in comparison with more vulnerable species whose distribution patterns have often changed during the last few decades because of human-induced habitat fragmentation (Huenneke 1991, Schmidt & Jensen 2000, de Lange & Norton 2004).

Genetic variability is a significant factor in the sustainability of plant populations (Wang et al. 2007). Any restriction on genetic variability reduces the evolutionary potential of species to adapt to changing environments (Jump et al. 2009). In the conservation of a species, knowledge of interspecies genetic variation may help assess the risk of extinction due to inbreeding and its evolutionary potential in a changing world (Hedrick 2001, Rahimmalek et al. 2009). The effective conservation of a vulnerable species largely depends on knowledge of patterns in its genetic variation. For example, the spatial structure of genetic variation can provide information for sampling strategies for either ex situ or in situ conservation (Torre et al. 2008).

Amplified fragment length polymorphism (AFLP; Vos et al. 1995) analysis is a more efficient way of detecting polymorphisms than other commonly used dominant marker methods [e.g. random amplified polymorphic DNAs (RAPDs), inter simple sequence repeats (ISSRs) and restriction fragment length polymorphisms (RFLPs) (Russell et al. 1997, Garcia et al. 2004)], and can be an attractive alternative to codominant markers such as microsatellites (or simple sequence repeats, SSRs) and single nucleotide polymorphisms (SNPs) (Meudt & Clarke 2007) in population genetic studies. To reveal the pattern of putative relationships of the localities that were investigated, sequencing of two chloroplast DNA (cpDNA) regions (*trn*L-*trn*F region and *trn*L-intron) were used. At the intraspecific level cpDNA may reflect the processes running within/between populations and may also reflect the pattern of gene flow. Sequencing or PCR-RFLP analysis of various coding/non-coding regions of cpDNA has been used in numerous population genetics studies of endangered species (e.g. Bettin et al. 2007, Gong et al. 2011; for more information see the review by Thompson 1999).

The objectives of the present study were: (i) to determine the genetic diversity and differentiation within and among *A. pancicii* populations throughout its natural range using AFLP markers; (ii) evaluate the relationships among fragmented populations of *A. pancicii* using the sequencing of two cpDNA regions, and (iii) propose a feasible conservation strategy taking into consideration the genetic patterns found.

Material and methods

Plant material

The distribution range of *A. pancicii* is highly fragmented and represents semi-dry grassland or steppe locations in southern Moravia (Czech Republic) and the eastern parts of Lower Austria and Burgenland (Austria). A list of the populations sampled with their basic geographical characteristics and the number of samples taken is presented in Table 1. Their geographical positions are shown in Fig. 1.

A total of 138 samples of fresh leaves were collected randomly from the 15 populations at the seven localities (Table 1). The samples were transported in a cooling box to the laboratory, treated with liquid nitrogen, and stored at –80 °C until DNA extraction. Ten samples were taken randomly from the whole area of the sampled population, with one exception. Population E was divided into two sub-populations according to the orientation of the slope to cardinal points (NW and SW respectively), with only five individuals sampled from each subpopulation. To avoid taking samples from the same cormone we collected the leaves from plants that were at least 20 cm from each other. The plants from which the samples were taken were marked with wire.

DNA extraction

Genomic DNA was extracted from leaf samples, each representing one individual, by the CTAB method (Kump & Javornik 1996) with minor modifications. The integrity and quality of the DNA was estimated using 1.8% agarose gels. Concentrations of DNA in the samples were determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA).

AFLP analysis

AFLP analysis was carried out according to the procedure of Vos et al. (1995), with modification after Kitner et al. (2008). In total six selective primer combinations were chosen to generate the AFLP profiles (Table 2). The products of amplification were separated on a 6%, 0.4-mm-thick denaturing polyacrylamide gel (0.5x TBE buffer) using the T-REX (Thermo Scientific Owl Separation Systems, Rochester, NY, USA) sequencing gel electrophoresis apparatus. Consequent silver staining was used for the visualisation of AFLP patterns.

CpDNA sequencing and sequence alingment

From each locality five randomly selected samples were used for sequencing the *trn*L-intron and *trn*F-*trn*L region using the primers *c*, *d* (for *trnL*-intron) and *e*, *f* (for *trn*F-*trn*L) given in Taberlet et al. (1991). PCR reactions were carried out using MangoTaq™ DNA Polymerase (Bioline, Luckenwalde, Germany) with the addition of BSA to a final concentration of 8 $\mu g \cdot \mu l^{-1}$ in the PCR reaction mix as described in Thines et al. (2010). The PCR program was set at an initial denaturalisation step of 3 min at 94 °C and 40 cycles of 50 s at 94 °C, 50 sec at 53 °C and 1 min 30 s at 72 °C, with a final extension step of 3 min at 72 °C in an MJ Research PTC-200 (MJ Research, Watertown, USA). The PCR products were purified (GeneElute PCR Clean-up Kit (Sigma-Aldrich Co., USA)) and sequenced with

Table 1. – Sampling details of the *Artemisia pancicii* populations studied. Country of origin: A – Austria, CZ – Czech Republic; ID = population code; n = number of samples taken for analysis, N = north, S = south, E = east, W = west, M = middle).

ID	Location	Country	Latitude (N)	Longitude (E)	Altitude (m)	n
A	Hundsheim (S)	A	48°07'25.7"	16°56'21.7"	366	10
B	Hundsheim (E)	A	48°08'15.8"	16°56'07.9"	366	10
C	Hundsheim (W)	A	48°07'57.9"	16°55'54.3"	356	10
D	Nickelsdorf	A	47°57'17.9"	17°03'22.1"	143	9
Ea	Neusiedl am See (NW)	A	47°56'37.1"	16°51'40.7"	151	5
Eb	Neusiedl am See (SW)	A	47°56'35.9"	16°51'41.0"	154	5
F	Neusiedl am See, Teichtal (W)	A	47°56'38.0"	16°51'54.8"	162	10
G	Neusiedl am See, Teichtal (M)	A	47°56'49.2"	16°52'09.7"	155	10
H	Neusiedl am See, Teichtal (E)	A	47°57'11.1"	16°52'26.1"	162	9
I	Spitzerberg	A	48°05'43.1"	16°58'34.9"	270	10
J	Čejkovice	CZ	48°55'23.9"	16°57'20.1"	221	10
K	Pouzdřany	CZ	48°56'49.9"	16°38'37.1"	291	10
P	Liščí kopec I	CZ	48°50'11.5"	16°33'47.9"	244	10
R	Liščí kopec II	CZ	48°50'17.3"	16°33'52.7"	256	10
S	Liščí kopec III	CZ	48°50'14.2"	16°33'58.3"	257	10

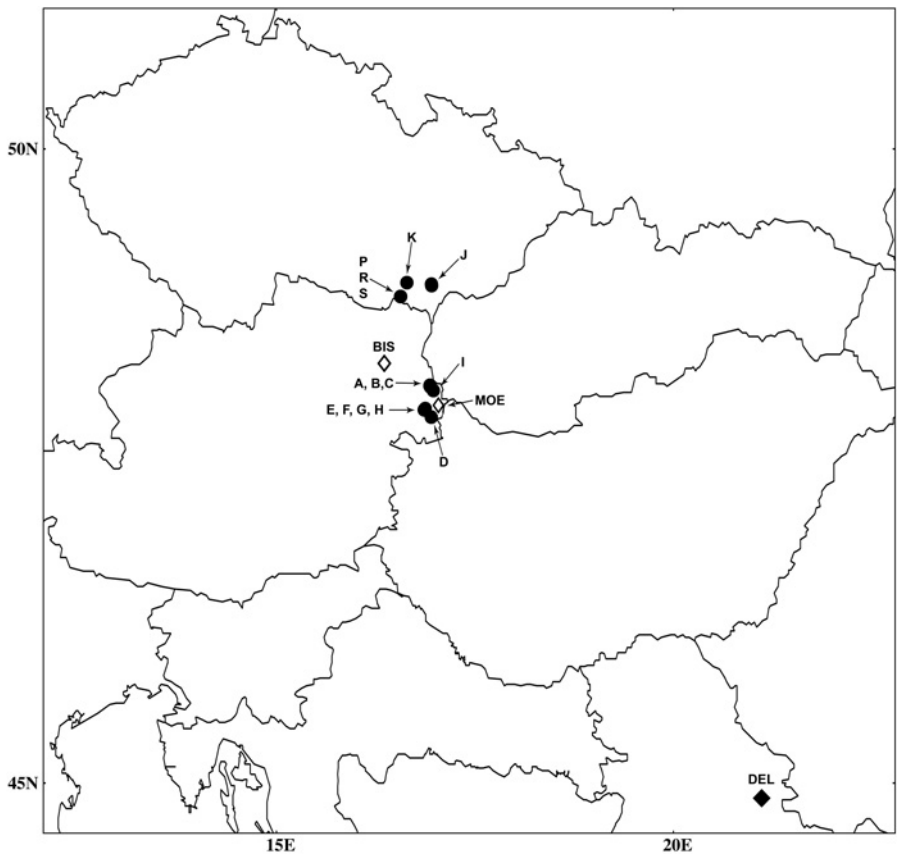

Fig. 1. – Current geographic distribution of *Artemisia pancicii* in Central Europe. Black full circles represent sampled populations (for population abbreviations and details see Table 1); black full diamonds represent the Serbian population "Deliblatska Peščara"; black empty diamonds represent Austrian populations that were not sampled (BIS = Bisamberg, MOE = Moenchhof).

Table 2. – Primers and primer sets for preamplification and amplification reactions with the total number of scored and polymorphic bands. Amplification sequence includes preamplification sequence in addition to three to five nucleotides listed.

Preamplification Primers Sequence			
EcoRI 5´– G ACT GCG TAC CAA TTC A –3´ MseI 5´– G ATG AGT CCT GAG TAA C –3´			
Amplification Primer Sets Sequences		No. bands	No. polymorphic bands
Set A	EcoRI primer E-AGC / MseI primer M-CAAC	83	74
Set B	EcoRI primer E-AGC / MseI primer M-CAATG	34	30
Set C	EcoRI primer E-AGC / MseI primer M-CAATC	58	54
Set D	EcoRI primer E-AGC / MseI primer M-CGATG	35	31
Set E	EcoRI primer E-ACC / MseI primer M-CGATC	85	66
Set F	EcoRI primer E-ACC / MseI primer M-CAACC	66	49

an Applied Biosystems 3730xl 96-capillary Genetic Analyser in the Laboratory of Molecular Cytogenetics and Cytometry at the Institute of Experimental Botany, AS CR (Olomouc, Czech Republic). Raw sequence data were aligned using the SeqMan module of the Lasergene program (DNASTAR Inc., WI, USA, version 4) to obtain the compiled sequences. Alignments and haplotype identification were performed with MEGA4 software (Tamura et al. 2007).

Data analysis

The visualised gels were scored for the presence (1) or absence (0) of bands. The binary matrix was constructed from primary data and subjected to FreeTree for cluster analysis (Pavlíček et al. 1999, Neighbour-joining tree, Dice similarity coefficient). The resulting cluster was visualised in TreeView (Page 1996). For the validation of particular branches bootstrap analysis was carried out using 1000 replicates (Felsenstein 1985). Principle coordinate analysis (PCoA, Jaccard's similarity coefficient) was performed with NTSYS-pc version 2.02 (Rohlf 1998). AFLP-SURV 1.0 was used to assess genetic variation within (H_w) and between (H_b) the populations that were investigated and for the calculation of the following population statistics: number of bands per population; proportion of polymorphic loci (*PLP*) at the 5% level; number of private bands; the expected population heterozygosity or Nei's gene diversity (H_e), the total gene diversity (H_t) and Wright's F_{st}. Because clonal reproduction is prevalent in *A. pancicii*, we used R-script AFLPdat (Ehrich 2006) for the calculation of genotype diversity (*GD*), number of genotypes (*NG*) and effective number of genotypes (*ENG*), based on the genetic structure in populations with clonal reproduction. Genotype diversity may be considered mathematically as equivalent to heterozygosity or Nei's gene diversity. The effective number of genotypes is analogous to the reciprocal of genotype diversity. If all the clones in the population were equally frequent, then the *ENG* would be the same as the number of clones; otherwise, the *ENG* is always less than the number of clones (Parker 1979). AFLPdat was also used for the calculation of *DW* or "frequency-down-weighted marker" values according to Schönswetter & Tribsch (2005). High values of *DW* are thought to indicate populations that have been isolated for a long time, while low values indicate populations that diverged recently. For the

calculation of frequency-downweighted marker values no adjustment for the number of individiuals was made and *DW* values were calculated for all the individuals within each population. Analysis of molecular variance (AMOVA) was performed using GenAlex 6 software (Peakall & Smouse 2006). Gene flow (N_m) among populations was estimated indirectly from the genetic structure of the population using Crow & Aoki's (1984) modified formulation of Wright's original (1951) equation: $F_{st} = 1/(4N_{m\alpha}+1)$, where $\alpha = [n/(n-1)]^2$ and n is the number of populations. To test an isolation-by-distance model Nei's genetic distance values (D; Nei 1978) were compared to the geographic distance (measured in km) between the populations using a Mantel test with 9999 randomizations (Mantel 1967). A significantly positive correlation indicates isolation by distance. To see how our data performed in model-based clustering we used mixture analysis in STRUCTURE 2.2 (Falush et al. 2007) and also in BAPS 3.2 (Corander et al. 2006). STRUCTURE uses a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals on the basis of multilocus genotype data. The input dataset was modified in such a way that the final data contained, in the case of clonal genotypes, only one individual with the genotype of the clone concerned. The analysis was set up for a recessive allele model and admixture model with independent allele frequencies. K was set to 1–17, with ten replicate runs for each K. The burn-in period was set to 100,000 iterations, after which a subsequent 1,000,000 MCMC iterations followed. The computation was carried out at the freely accessible Bioportal of the University of Oslo (www.bioportal.uio.no). R-script Structure-sum-2009 (Ehrich 2006) was used to summarize the output files: the calculation of similarity coefficients (SC) between replicate runs for the same K (calculated accordind to Nordborg et al. 2005, with approach described in Ehrich et al. 2007), means of the posterior log probability [mean$L(K)$] and a quantity based on the second-order rate of change of the likelihood function with respect to K (ΔK) (as denoted in Evano et al. 2005). Additionally, two programs, CLUMPP (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004), were used for summarizing the STRUCTURE outputs and figuring the clustering graphically. Bayesian clustering implemented in the BAPS software allows the genetic structure of populations to be inferred on the basis of an estimate of the highest probability partition and the assigment of individuals to one of K populations. In BAPS both the allele frequencies and the number of genetically divergent groups are treated as random variables. For estimating the optimal number of clusters we used the "clustering of individuals" module. In the case of clonal genotypes, the input data contained only one genotype of the clone concerned. The analysis was run ten times for K set to a maximum 1–17.

To check the reliability of the AFLP analysis, the amplification for each primer combination with the whole sample set and, additionally, amplification of randomly choosen samples (from two to three samples from each population, together with samples with uncertain genotypes) was repeated. The gels were scored independently. In the last step, both results of scoring were compared and checked for the number of markers, intensity of markers and relative position of markers. In the final binary matrix only verified markers (present in the original and repeated amplification) were used. The error rate was calculated as the difference in the total number and the number of fragments used in the final matrix.

To evaluate the relationships among *A. pancicii* populations, a cpDNA haplotype network was constructed in the TCS program version 1.12 (Clement et al. 2000).

Results

Genetic variability

A total of 138 plants of *A. pancicii* from 15 populations were analysed using six AFLP primer combinations (Table 2), which generated 359 bands, of which 301 were polymorphic (83.8%) and the mean number of fragments per individual was 164.5. The number of bands and percentage of polymorphic bands per primer combination are summarised in Table 2. Replication of the analysis revealed the high reliability of AFLP, with an error rate of 1.1% (it represents four markers). The total gene diversity was high ($H_t = 0.248$). AFLP polymorphism varied within (in terms of *PLP* and H_e indices) and across the populations (Table 3). The lowest genetic variability was observed in population R, with *PLP* = 1.1% and $H_e = 0.008$. The highest genetic variability was in population D, with *PLP* = 59.6% and $H_e = 0.152$. The genetic variability indices for all populations are summarized in Table 3.

Population genetic structure

Significant genetic differentiation among the populations was observed. The AFLP-SURV derived genetic differentiation measure, $F_{st} = 0.75$, was significantly different from zero. Nei's genetic distance (*D*) ranged from 0.0785 (between populations I and P) to 0.3109 (populations B and J) (data not shown), suggesting a high level of genetic differentiation among pairs of populations. Nevertheless, the highest values (over 0.2900) occurred between populations A, B, Ea, Eb and K, J. Proportioning of total gene diversity and AMOVA analysis (Table 4) also revealed a high ratio of variation among the populations ($H_b = 0.186$; 82%) compared to the little genetic differentiation within *A. pancicii* populations ($H_w = 0.062$; 18%). The gene flow (N_m) among the populations was estimated as 0.083 individuals per generation, indicating an extremely restricted gene flow caused by limited pollen and seed dispersal between populations.

The Mantel test did not reveal a significant correlation between the genetic and geographic distance matrices, whereas the P value was marginally significant (r = 0.1820, P = 0.075). When the Mantel test is performed after the subseparation of the localities into three groups according to their geographical distribution, significant negative values were revealed for the area of Hundsheim (A, B, C, I) and the Czech localities (P, R, S, J, K) (r = –0.419, P = 0.042; and r = 0.643, P = 0.056 respectively). There was no significant support from the Mantel test for localities Ea, Eb, F, G, H from the Neusiedl am See area (r = –0.015, P = 0.447). These negative values mean that the closer the populations are, the greater the genetic distance is observed.

To reveal the relationships among the populations in detail, neighbour-joining analysis was performed on the basis of the Dice similarity coefficient. The prominent grouping of samples was based on their origin, as can be seen in the unrooted tree (Fig. 2). Each population that was studied formed one cluster, with the exception of the subpopulations Ea and Eb from the Neusiedl am See locality, which are disjoined and clustered separately in different clusters with the Hundsheim populations. However, high bootstrap support was observed only at the intrapopulation level, with weak support for the basic branching of the neighbour-joining tree.

Further detailed visualisation of the relationships within and between the populations was performed using principal coordinate analysis (PCoA, Fig. 3). On the PCoA plot

Table 3. – Summary of the genetic variability indices of the populations investigated based on allele frequencies for 359 AFLP loci. n –number of samples, NB – number of bands, N_{Pol}, N_{Pri} – number of polymorphic and private bands, PLP – proportion of polymorphic loci, H_e, SE – estimated heterozygosity with standard errors, DW – frequency-down-weighted marker values, GD – genotype diversity, GN – number of genotypes, ENG – effective number of genotypes (according to Parker 1979). Population codes are the same as in Table 1.

Population	A	B	C	D	Ea	Eb	F	G	H	I	P	R	S	J	K	Mean
n	10	10	10	9	5	5	10	10	9	10	10	10	10	10	10	–
NB	198	207	177	214	152	149	153	195	165	195	212	166	201	211	190	185.67
N_{Pol}	48	72	34	96	7	11	18	48	16	78	91	4	32	43	19	41.13
N_{Pri}	9	4	3	5	1	3	2	4	0	3	1	2	1	5	7	3.33
PLP (%)	13.4	20.1	9.5	59.6	1.9	3.1	5.0	13.4	4.5	21.7	59.1	1.1	8.9	12.0	5.3	11.46
H_e	0.065	0.087	0.044	0.152	0.018	0.025	0.028	0.077	0.025	0.119	0.142	0.008	0.040	0.067	0.029	0.036
SE	0.006	0.006	0.005	0.009	0.003	0.003	0.004	0.006	0.004	0.008	0.008	0.002	0.004	0.006	0.004	0.005
DW	2.8	2.7	2.2	2.6	1.6	2.0	1.4	2.2	1.6	1.8	1.9	1.7	2.2	3.0	2.6	2.2
GD	0.98	0.98	0.76	0.97	0.60	0.60	0.78	0.87	0.56	0.76	0.96	0.00	0.84	0.96	0.65	0.75
NG	9	9	5	8	2	2	4	6	3	5	8	1	6	8	4	5.3
ENG	8.33	8.33	3.13	7.36	1.92	1.92	3.33	4.55	1.98	3.13	7.14	1.00	4.17	7.14	2.38	4.39

Table 4. – Results of analysis of molecular variance (AMOVA) within and among populations of *Artemisia pancicii*; d.f. – degrees of freedom, SSD – sum of squared deviation.

Source of variation	d.f.	SSD	Est. var.	Percentage total	P-value
Among populations	14	4719.218	35.863	82%	
Within populations	123	976.144	7.936	18%	0.819

a clear separation of the localities A and J is obvious. The rest of the populations fall within one large cluster separated from the rest of the samples along the second axis. The populations from this cluster show some substructure along the first and third axis. The most isolated clump is formed by populations C, B, Eb, Ea, H, K and F, while populations G, S, P, I, D and R are more dispersed within the cluster. The first two axes of the PCoA plot are nearly at the same percentage and explain only 18% of the overall variability.

The results of Bayesian clustering in STRUCTURE showed that the mean $L(K)$ increased up to $K = 2$, while for a higher K the likelihood flattens out. ΔK also indicates the maximum values for $K = 2$ and $K = 5$ with the second highest value, suggesting that the best partitioning is into two (or alternatively five) clusters (Evano et al. 2005). Replicate runs did not give stable results for any K. The similarity coeficient for $K = 2$ was 0.26 and for $K = 5$ it was 0.77. The similarity coefficient for $K = 5$ was the highest recorded, but the one for $K = 2$ was lower than for the other values of K; however, Ks higher than $K = 5$ produced empty groups (e.g. for $K = 8$ six runs out of ten produced empty groups). For $K = 2$, altogether three possible ways (clusterings) of placing samples into different groups of similar genotypes were found, and for $K = 5$ ten replicate runs also produced three different clusterings. For $K = 2$, the clustering supported by the highest mean $L(K)$ was two clusters and localities A and J are separated from the rest (Fig. 4). This is consistent with the PCoA separation of localities along the 2nd axis. Similarly, for $K = 5$, the clustering with the highest mean $L(K)$ produced discrete clusters for localities A, B, C, J and the rest of the localities grouped in one large cluster (Fig. 4). This is also in partial concordance with the genetic variability indices (Table 3).

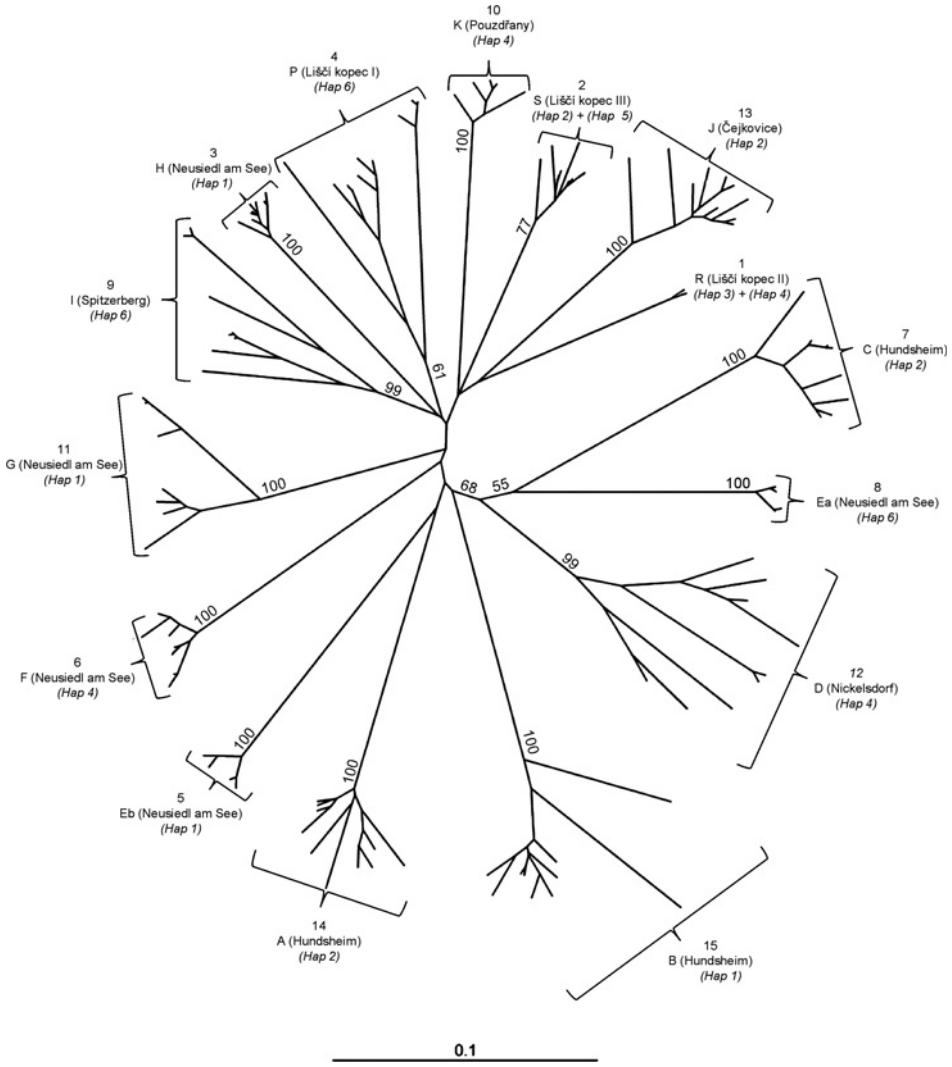

Fig. 2. – Unrooted neighbour-joining tree based on Dice coefficient of similarity of 15 *Artemisia pancicii* populations inferred from AFLP data. Bootstrap values > 50 are shown above the branches. Results of Bayesian clustering are indicated above the population names as numbers of the corresponding cluster. CpDNA haplotypes are given below the population names.

The result of BAPS clustering revealed 15 different clusters (Fig. 2) in optimal partitioning with the highest log marginal likelihood (probability = 1). The other possibilities of clustering with lower probabilities did not clarify the situation and were not further evaluated. The individuals were assigned to the clusters according to their population origin. This result was stable in all replications.

In our dataset, the number of private markers ranged from nine to zero (Table 3). The highest numbers were found in the Austrian populations from the Hundsheim area (A – nine private markers, D – five, B – four, and C – three) and in the Czech populations

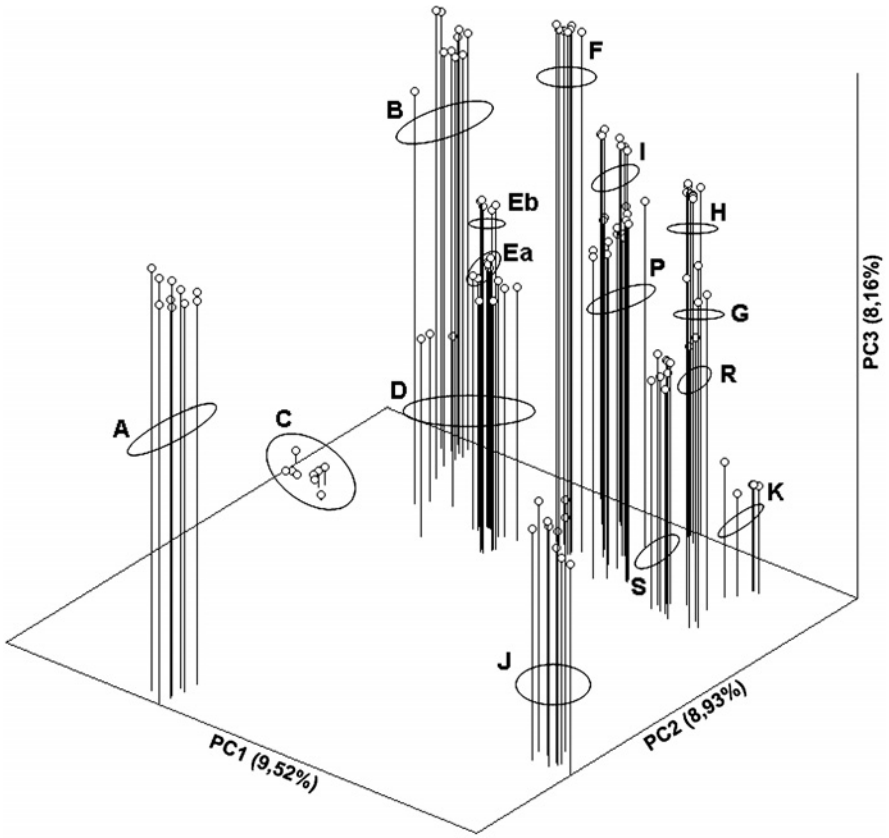

Fig. 3. – Principal coordinate analysis of pair-wise Jaccard's similarity matrix of 138 *Artemisia pancicii* samples. The first three principal coordinates accounted for 26.61% of the total variation. Population abbreviations are those in Table 1.

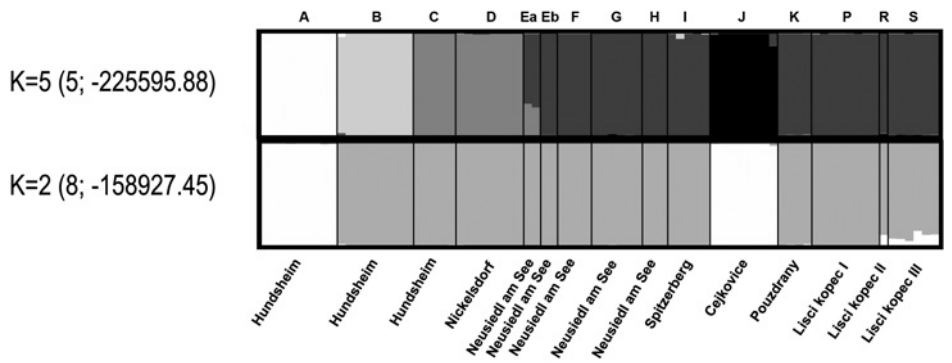

Fig. 4. – Results of Bayesian assignment of *Artemisia pancicii* (80 individuals, 15 populations) into clusters using the STRUCTURE program. Each vertical bar represents one individual with the colour representing the assignment probability to different clusters. Only the most frequent clusterings obtained for $K = 2$ and $K = 5$ are shown, with the number of replicate runs producing the partition and the mean $L(K)$ value. The names of the populations are displayed below and their abbreviations above the graphic.

(K – seven private markers, J – five). The *DW* values are low and partially correlate with the presence of private markers. The highest *DW* values were found in the Czech population J (*DW* = 3.0) and in the Hundsheim populations A and B (*DW* = 2.8 and 2.7 respectively).

The investigation of clonality in the *A. pancicii* populations revealed higher levels of uniformity than expected in a sexually reproducing species. Individuals with genotypes differing by four markers (correspond to an error rate of 1.1%) were considered as a single clone. The lowest number of genotypes was observed at locality R (one genotype), locality Ea and Eb (two genotypes) and locality H (three genotypes) (Table 3). The highest genotype diversity was recorded for the Hundsheim populations (0.98 at A, B; 0.97 at D) and the Czech populations at the Liščí kopec locality (0.96 at P, J; 0.84 at S). The values of the effective number of genotypes correlated with genotype diversity, so the highest values were in populations A, B, D, P and J.

The *trn*L-*trn*F cpDNA region and *trn*L-intron were sequenced for all 15 *A. pancicii* populations analysed by AFLP. Altogether seven single-point mutations (Table 5) were revealed, four SNPs in the *trn*L-*trn*F region and three SNPs in the *trn*L-intron. A total of six haplotypes were identified when both regions were combined (Hap1–Hap6; Table 5). In general, there was only one genotype at each locality, with two exceptions, localities R and S, where there was one additional haplotype (R1 and S1 in Table 5). The results of the TCS analyses (Fig. 4) mirror the neighbour-joining tree topology in the sense of the absence of a clear geographical separation of localities. The biggest outgroup probability was assigned to the ancestral-like haplotype Hap1 (localities "B, Eb, G, H"), with Hap6 (localities Ea, I, P) as the most progressive one.

Table 5. – Haplotype identification and position of SNPs in the *trn*L-*trn*F intergenic spacer and *trn*L-intron chloroplast DNA (cpDNA) haplotypes. Population codes as in Table 1 (R1, S1 are minor haplotypes at appropriate localities R and S).

Population/consensus	T	C	C	T	C	T	G	Haplotype
B	.	T	.	.	.	G	T	Hap1
Eb	.	T	.	.	.	G	T	Hap1
G	.	T	.	.	.	G	T	Hap1
H	.	T	.	.	.	G	T	Hap1
A	.	T	G	.	.	G	T	Hap2
C	.	T	G	.	.	G	T	Hap2
J	.	T	G	.	.	G	T	Hap2
S	.	T	G	.	.	G	T	Hap2
R1	.	T	G	.	.	.	.	Hap3
D	.	.	.	.	.	.	.	Hap4
F	.	.	.	.	.	.	.	Hap4
K	.	.	.	.	.	.	.	Hap4
R	.	.	.	.	.	.	.	Hap4
S1	.	.	.	.	.	G	T	Hap5
Ea	G	.	.	G	T	G	.	Hap6
I	G	.	.	G	T	G	.	Hap6
P	G	.	.	G	T	G	.	Hap6
Position of SNPs on sequences	*trn*L-*trn*F				*trn*L-intron			
Joined sequences (fragment length 836bp)	56	153	169	290	481	525	595	
*trn*L-*trn*F (fragment length 422bp)	56	153	169	290	–	–	–	
*trn*L-intron (fragment length 414bp)	–	–	–	–	59	103	173	

Discussion

Genetic variability

Artemisia pancicii is a very rare species occurring at only a few localities in three countries in Central Europe (Fig. 1). The denomination "rare species" is typically assigned to species with low levels of genetic variation and in this respect it is so classified in several reviews of large numbers of isozyme studies (Chung & Kang 1996, Allphin et al. 1998, Gitzendanner & Soltis 2000, Lienert et al. 2002). There are not many recent populations of *A. pancicii*. The richest populations probably consist of thousands of ramets. So the expectancy of low variability in such populations is justified. The observed values of genetic variability were between 0.142 and 0.008 (Table 3). Higher values of genetic variability $H_e > 0.100$ were recorded for three populations (the values for all other localities were lower $H_e < 0.100$). Two of them are in Austria (D, I) and one (P) in the Czech Republic. The population with the lowest variability ($H_e = 0.008$) was the R population (Liščí kopec II). The higher value of variability may reflect the condition and number of individuals and this may suggest the presence of recent sexual reproduction at this locality. In a study dealing with population dynamics and genetic variability of population P (Kitner & Majeský, unpublished data), the genetic variability ($H_e = 0.031$) recorded was also quite low. This value is very similar to the mean value observed in this study. The lower value for the neighbouring population R may reflect the sterile status of this population and the high density of individuals of clonal origin. Torrell et al. (1999) record a higher genetic variability based on isozymes in two populations (H_e = 0.426 and 0.371 respectively) of another rare *Artemisia* species (*A. molinieri*). In general, isozyme-derived estimations of indices of genetic variability detect much lower levels of polymorphism (Weising et al. 2005) compared to estimates based on DNA markers (AFLP). This contrast between the results recorded in studies on *A. pancicii* and *A. molinieri* is explained by the authors of the study on the not genetically depauperate (stunted) *A. molinieri* (Torrell et al. 1999). They found generally higher values of revealed genetic indices, compared to similar endemic taxa and/or a dicotyledonous, perennial of anemophilous and anemochorous character. The Wright's F_{st} recorded in this study suggests highly differentiated populations (F_{st} = 0.75). This corresponds to the genetic differentiation of another rare xerothermic Central European plant species, *Globularia bisnagarica*, a species ecologically similar to *A. pancicii*, but growing in different steppe habitats, of which 27 populations were characterized by high genetic differentiation $F_{st} = 0.53$ (Honnay et al. 2007). In Torrell et al. (1999) the F_{st} values were lower (varying from 0.001 to 0.123 for different isozyme systems).

The high genetic differentiation among populations and genetic homogeneity within populations could have arisen as a result of low gene flow. A migration rate of 0.5 is considered sufficient to overcome the diversifying effects of random drift (Ellstrand & Elam 1993). In our study, the estimated gene flow for *A. pancicii* ($N_m = 0.083$) is lower than the average value reported for mixed-mating or out-crossed animal-pollinated species (0.727 and 1.154 respectively) detected by allozyme analysis (Hamrick & Godt 1989) or the high N_m estimates (4.21) for fragmented populations of *Anthyllis vulneraria* attributed to seed dispersal by sheep (Honnay et al. 2006). Generally, high gene flow maintains genetic and phenotypic homogeneity, whereas low gene flow leads to isolation by distance and drift (Alleaume-Benharira et al. 2006). Gene flow can counterbalance the reduction in genetic variability of individual populations by bringing in new genetic material through seeds and pollen (Young

et al. 1996), while random genetic drift is expected to increase genetic differentiation among populations in the absence of gene flow (Harrison & Hastings 1996, Schaal & Leverich 1996). The current populations of *A. pancicii* are fragmented and gene flow between nearby populations is so low that it cannot be considered as a source of population diversification.

Population structure

Generally, the genetic population structure of a species is affected by a number of evolutionary factors, including its mating system, gene flow, seed dispersal and mode of reproduction, as well as natural selection (Hamrick & Godt 1989). Habitat fragmentation is generally recognised as a major threat to plant population persistence (Mix et al. 2006). A progressive reduction in the number of suitable habitats has led to smaller populations separated by often unbridgeable, unsuitable habitats. As a result, populations face the consequences of demographic, environmental and genetic stochasticity (Schaffer 1987), which increases the probability of extinction (Lande 1993, Schemske et al. 1994).

The detection of private alleles in some populations further supports our hypothesis of the independent history of local populations of *Artemisia pancicii* in fragmented habitats accompanied by limited, or even absent, gene flow over a very long period of time (e.g. Slatkin 1987, Avise 1994). A low number (0-3) of AFLP fragments unique to a particular population indicates too short a time for the development of unique fragments through mutation, as reported in fragmented populations of the endangered perennial steppe plant *Iris aphylla* (Wróblewska et al. 2010). In our study, a higher number of private alleles (5–9) and high frequency-downweighted marker (*DW*) values were found at the Hundsheim areas and Czech localities (Table 1). Both the presence of a higher number of private markers and higher values of *DW* may suggest populations with a longer independent history of existence without or with just restricted gene flow between particular populations, because of the time they have for the accumulation of mutations (which is reflected in the higher rates), in contrast to the lower values expected in recently established populations, which can be used for differentiating among these populations (Mráz et al. 2007, Kučera et al. 2008, Paun et al. 2008).

Wormwoods are species with short-distance dispersal mechanisms. They produce small achenes which germinate in close proximity to the mother plant or can be dispersed over short distances by the wind (Friedman & Orshan 1975, Whitacre & Call 2006). In the case of unsuccessful (as a result of problems with reproduction) or even successful seed setting and the lack of some artificial recent long-distance dispersal event, it could lead to a clear separation of individuals from all populations into clusters according to their geographical origin, as is partially visible in the neighbour-joining tree (Fig. 2), which indicates the independence of all the Austrian and Czech populations. However, the clustering of these populations does not strictly follow the geography. This is also evident from the sequencing of cpDNA regions, where no geographically based pattern of haplotype distribution was found (see Table 5 and Fig. 4). The separation of compact clumps of samples both in the neighbour-joining tree and on the PCoA plot indicates high uniformity and low genetic variability at the intrapopulation level. Both molecular methods separated the Neusiedl am See subpopulations Ea and Eb, which are in fact divided only by a short path. It could be explained as having its origin in the presence of different gene pools from which different types of clones have originated, with distinct demands on niches.

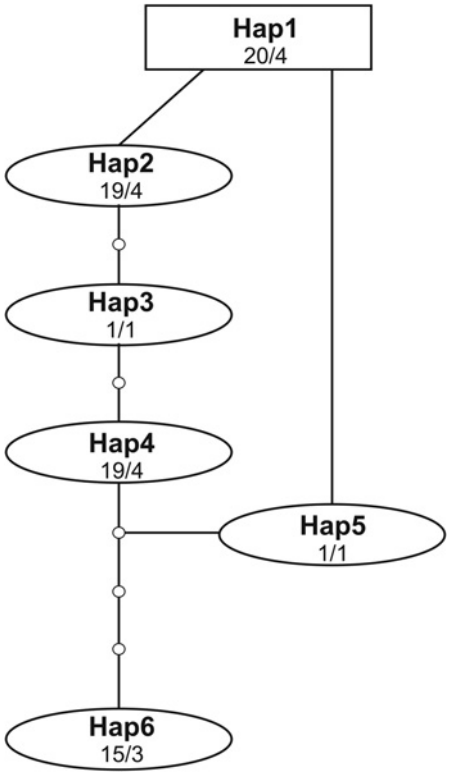

Fig. 5. – Statistical parsimony network depicting genealogical relationships between the haplotypes of two chloroplast regions. Haplotype identification (Hap1-Hap6), the number of individuals with the number of populations is given in each oval or box, which represents the putative ancestral haplotype. Dots represent inferred intermediate haplotypes that are extinct or were not sampled.

From the topology of the neighbour-joining tree it is not possible to identify some relevant structure of investigated populations. While in the neighbour-joining tree the partial separation of the Austrian from the Czech populations (Fig. 2) occurred on the PCoA (Fig. 3) plot and haplotype network (Fig. 5), the separation is not obvious. All the axes of the PCoA graph provide nearly the same percentage of explanation and only 27% of all the variability can be explained by the first three axes. Despite the grouping of the majority of the Austrian and Czech populations in one cluster an inner substructure within this cluster can be found, but it is not possible to demarcate some line of division between them on the basis of geography. All the populations studied are quite different and seem to be isolated from each other and to form an independent unit in space and time, with very low gene flow between localities and also between populations within localities. This is also supported by the result of the Bayesian clustering, which did not help resolve the structure among the *A. pancicii* populations. STRUCTURE outlined two possible scenarios with the separation of two or five clusters (Fig. 4). These clusters partially followed the separation of populations in the PCoA analysis and separated those populations with high values of genetic variability. However, the BAPS clustering was different, with the identification

of fifteen different clusters (Fig. 2) without a mixed pattern. The geography also does not explain the pattern of population structure and the relatedness of populations is independent of geographical distance. The status of the current populations is most probably the reflection of the historic postglacial distribution of this taxon and their current fragmented relict status with very restricted gene flow. The environmental conditions at the time of the origin of *A. pancicii* differ from those of today and are ecologically suboptimal (Danihelka 1995). *Artemisia pancicii*, together with other members of the subsect. *Laciniatae*, are typical elements of steppe or wooded-steppe formations (Danihelka 1995), which spread into Central Europe at the end of the Last Glacial Maximum (LGM) and the beginning of the postglacial (Ehrendorfer 1964). Further climatic changes, such as warmer and moister weather, contributed to the development of deciduous woodlands, which are unfavourable for steppe plants and resulted in a decline in their abundance (Danihelka 1995). The most appropriate diversifying forces for *A. pancicii* populations seem to be genetic drift with local selection for the most suitable genotypes.

Clonality

The findings of the present study, together with field observations may suggest that *A. pancicii*, at the localities studied, reproduces mainly by clonal growth rather than generative reproduction. In the majority of populations sterile shoots prevail and fluctuations in the number of ramets were observed, however, without statistical support (L. Gillová, unpublished data). In all cases the number of different genotypes was lower than the number of analysed samples in any population (Table 3). More than five different genotypes were present only in populations A, B, D, G, P, S and H, whereas the most clonal was the Czech population R (Liščí kopec II), where all the individuals that were analysed have the same genotype. This population is considered to be the most clonal with no flowering individuals observed over a period of five years (L. Gillová, unpublished data). This population might represent the remains of a previously larger historical (meta) population at Liščí kopec, where fragmentation led to the dispersal of several genotypes to different places at the original locality and the most successful genotype survived and formed this clonal population. The situation in population P (Liščí kopec I) is quite different. This population is considered to be vital because of the possibility of sexual reproduction. In population P (Liščí kopec I), genetic variability as a result of microhabitat preferences might occur, meaning more frequent flowering (with possible seed set) and the presence of different genotypes (L. Gillová, unpublished data). The low number of genotypes found in the Austrian locality of Neusiedl am See (populations Ea and Eb) may reflect that the sample from both these populations consisted of only five individuals. However, it is still interesting that such a high degree of clonality in a sexual species is recorded, even if the sampling was performed at random across the whole population.

Our previous observations of the reproductive patterns in *A. pancicii* suggest that some type of sexual incompatibility is present in this species. Brewbaker (1957, 1967) found that species with trinucleate pollen have mainly sporophytic systems of self-incompatibility and members of the *Asteraceae* have trinucleate pollen. Thus we may expect a sporophytic system of self-incompatibility to be present in the genus *Artemisia*. Vallejo-Marín & O'Brien (2007) found that clonal reproduction is strongly associated with self-incompatibility: the absence of clonality is widespread among self-compatible taxa, while

all self-incompatible species reproduce clonally. Vegetative (clonal) reproduction, which occurs concurrently with sexual reproduction in a large proportion of flowering plants (Richards 1986), may provide an alternative way of assuring reproduction. In this sense, clonality may act as a mechanism of uniparental reproduction comparable to selfing (Nagylaki 1976, Charlesworth 1980) that allows a genotype to persist and increase in numbers under conditions of pollen limitation (Baker 1955, Dole 1992, Holsinger 2000). For species that are often subjected to pollen limitation, such as colonising or endangered plants, clonality may thus relieve the selective pressure favouring the breakdown of outcrossing mechanisms. An isozyme study of *A. molinieri* indicates that vegetative propagation is the main means of propagation in this species (Torrell et al. 1999). On the one hand, long-lived plants with clonal propagation tend to have remnant population systems, in which many local populations persist over periods long enough to bridge unfavourable phases of successional development and intervening periods of favourable conditions (Eriksson 1996). On the other hand, the presence of a high degree of clonality in *A. pancicii* populations, together with the reduced level of sexual reproduction (even self-fertilisation) and the absence of well-developed seeds detected in this study, may suggest that *A. pancicii* reproduces by apomixis. Although apomixis is not rare in the *Asteraceae* (van Dijk 2003), in the literature there is only scant information on apomixis in the genus *Artemisia* (for information on apomixis in the *Asteraceae* and the genus *Artemisia* see Noyes (2007) and the references therein). Chiarugi (1926) reports the development of aposporous and diplosporous female gametophytes in *Artemisia nitida*; however, other records on apomixis in *Artemisia* are lacking and therefore it requires further study.

A decreased rate of sexual reproduction is also reported by Torrell et al. (1999). Vymyslický investigated the composition of the soil at several localities where *A. pancicii* occurred and concluded that the species grows on neutral or light alkaline soil with an adequate to high content of potassium, magnesium, calcium and a humus layer. In contrast, the phosphorus content of the soil was low and this could be one of the limiting factors for generative reproduction (unpublished data). A test of the viability of pollen grains revealed reduced viability of pollen; only 10% to 30% of the pollen grains of *A. molinieri* are viable (Torrell et al. 1999). Skálová records that only 18.3% of the pollen grains of *A. pancicii* are viable (unpublished data). Gardeners at the Viennese Faculty Centre of Biodiversity have not been able to propagate *A. pancicii* from seed (personal communication). Torrell and coworkers also observed an extremely low germination rate of achenes of *A. molinieri*, even of morphologically well developed seeds (Torrell et al. 1999). The viability of pollen and achenes is affected by various environmental and storage conditions and the result of the viability test could be influenced by these factors, but nevertheless observations in the field (at least in the case of *A. pancicii*) confirm an overall decreased outcrossing rate, at least in some populations. In the Czech and Austrian populations no viable achenes are recorded (Holub & Grulich 1999, Bulová 2002) and accordingly we only have evidence of vegetative reproduction. Seed was collected in 2006 and 2008 at the Pouzdřany and Liščí kopec I localities and analysed in the laboratory. Unfortunately, none of the seeds germinated. This might also be due to the fact that none of the achenes were fully ripe.

Implications for conservation

The relict status and limited geographical distribution of populations of *A. pancicii* (Fig. 1), limited ability to spread and persistence in small populations make this species very interesting for, and dependent on, protective conservation. This study shows that the genetic variability within populations is very small with the whole-world population of *A. pancicii* consisting only of several polycormons. It is neccessary to carry out strict conservation with appropriate habitat management (e.g. mowing, grazing, or fire management) of current populations to ensure the long-term protection of *A. pancicii*.

Reduced genetic variability reduces the evolutionary potential of species to adapt to changing environments. Even if we accept the assumption that naturally rare species have more extinction-resistant life history traits (Kunin & Gaston 1993, Gaston & Kunin 1997), there is still a high risk of extinction in such fragmented and small populations as a result of catastrophes or environmental stochasticity. Furthermore, there are increasing numbers of reports that contradict the hypothesis that naturally rare species are more extinction-resistant (Harrison et al. 2008).

Artemisia pancicii populations are endangered by shrub invasion as a consequence of the absence of management and also by the eutrophication of the localities where this species occur (Holub & Grulich 1999, Ellmauer 2005). The data from demographic studies (L. Gillová, unpublished data) show that this species at localities with appropriate conservation management (such as mowing, grazing, or fire management) are more abundant, stable and have a greater number of flowering ramets. This can also be observed at Nickelsdorf, where there is one of the most diverse populations, which has been grazed by five cows for some time. Monitoring of pastures indicates that this method of management is suitable for conserving this species' rare occurrences and holding back shrubs, which also favourably affects several other rare species occurring in these habitats (Nagler 2010). However, it is noteworthy that cattle seem to prefer inflorescences to ground rosettes. Although at Nickelsdorf grazed plants are capable of growing inflorescences in late September after grazing ceases, the grazing period should not extend too far into summer in order to provide enough time for proper anthesis. An appropriate period is three months, for example from April to June. The precise number of animals depends on the pasture area and the duration of the grazing and should not exceed the values that would destroy the population of the plant. There is no difference between the effects of cow and sheep grazing at this locality, but this might be due to the relatively short period of investigation, which was only one year (Nagler 2010). Cow grazing at a steppe locality is very unusual; its use at Nickelsdorf is a rare occurrence but possible because of the large area of habitat at this locality. The majority of the *Artemisia* localities are small and steep and the use of the grazing by cows can cause problems (eutrophication, erosion etc.). Extensive grazing by sheep or goats in the spring or early summer months seems to be more appropriate. However, the selection of an appropriate "grazing" management is a matter for a detailed study, and the populations should be monitored to determine whether the management results in higher fitness.

Moreover, this species inhabits communities from open grasslands to shrub and wood associations. Although medium shade does not seem to negatively affect the number of inflorescences, this species does not flower in heavy shade (Nagler 2010). As this species seems to be tolerant of degradation (Wendelberger 1959) and its habitat is resilient to perturbance by long term extensive pasture by humans, grazing may be considered an

appropriate way of managing this species and the character of the general landscape. Other appropriate methods of management are mowing or fire. The number of flowering ramets recorded at Liščí kopec one year after mowing was greater (L. Gillová, unpublished data) and the number of ramets after a fire at Pouzdřany in 1988 greatly increased (Danihelka 1995).

Conclusions

Our results show that the *A. pancicii* populations are highly differentiated, with limited gene flow between populations and a prevalence of clonal growth and little sexual reproduction. We did not find any hierarchical structure in the relationships of the population, so to maintain the highest possible variability within the species each population can be seen as an independent target for conservation. With long-term demographic monitoring data, we can make a relatively realistic simulation model of population dynamics, preferably including information on levels of environmental stochasticity (Oostermeijer et al. 2003). Future studies of inter-population outcrossing could help to answer the question of whether artificial gene flow among populations would help to increase the populations' genetic variability. However, the fitness consequences of such trials are not necessarily beneficial, because previous studies have not only found increased offspring fitness after interpopulation crosses (Luijten et al. 2002, Oostermeijer et al. 2003), but also outbreeding depression after crosses among plants from different neighbourhoods or populations (e.g. Waser & Price 1994, Fischer & Matthies 1997, Fenster & Galloway 2000). Integrated management can also be ensured with ex situ collections of whole plants, pollen, vegetative propagules, tissue, or cell cultures. In situ conservation should include population monitoring and experiments to determine which method of management achieves the best results.

Acknowledgements

We would like to thank S. Gill (Olomouc), V. Grulich (Brno), F. Krahulec (Průhonice), R. J. Vašut (Olomouc) and two anonymous reviewers for their constructive comments on the manuscript, as well as F. M. Grünweis (Vienna) for his assistance with plant sampling. This work was financed by the Ministry of Education, Youth, and Sports of the Czech Republic (projects MSM 6198959215, MSM 2629608001, 2B06178, IGA Prf-2010-001).

Souhrn

Panonský endemit *Artemisia pancicii* (pelyněk Pančičův) patří v evropském měřítku k nejvzácnějším rostlinám. Jeho areál rozšíření je značně fragmentován a v současnosti se jeho výskyt uvádí z deseti lokalit z území České republiky, Rakouska a Srbska. V této studii prezentujeme výsledky analýzy genetické diverzity patnácti českých a rakouských populací *A. pancicii* získané metodou AFLP a sekvenací dvou oblastí chloroplastové DNA. Zjištěná vysoká úroveň celkové mezipopulační genetické diverzity ($H_t = 0.248$) a poměrně vysoká hodnota Wrightova fixačního indexu ($F_{st} = 0.75$) ukazují na fixaci alel v jednotlivých, víceméně vzájemně geneticky odlišných populacích. Analýza molekulární variance (AMOVA) prokázala, že větší podíl genetické diverzity je lokalizován na mezi-populační úrovni ($H_b = 0.186$; 82%), zatímco úroveň genetické diverzity jednotlivých populací je výrazně nižší ($H_w = 0.062$; 18%). Hlavními příčinami nízké variability studovaných populací *A. pancicii* je pravděpodobně omezený gene flow a genetický drift. *Artemisia pancicii* je druh konkurenčně velmi slabý, pouze populace na lokalitách s vhodným ochranářským managementem (pravidelné kosení, pastva či řízené vypalování) mají větší počet jedinců s vyšším podílem kvetoucích prýtů a vykazují i vyšší genetickou diverzitu. Pro ochranu a zachování dostatečného počtu populací je nutné začít s pravidelným ochranářským managementem.

References

Alleaume-Benharira M., Pen I. R. & Ronce O. (2006): Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. – J. Evol. Biol. 19: 203–215.

Allphin L., Windham M. D. & Harper K. T. (1998): Genetic diversity and gene flow in the endangered bear poppy, *Arctomecon humilis* (*Papaveraceae*). – Am. J. Bot. 85: 1251–1261.

European Topic Centre on Biological Diversity (2009): Habitats Directive Article 17 reporting: *Artemisia pancicii*. – European Environment Agency, Brussels, URL: [http://eea.eionet.europa.eu/Public/irc/eionet-circle/habitats-art17report/library?l=/datasheets/species/plants/plants/artemisia_panciciipdf/_EN_1.0_&a=d]

Avise J. C. (1994): Molecular markers, natural history and evolution. – Chapman and Hall, New York.

Baker H. G. (1955): Self-compatibility and establishment after "long-distance" dispersal. – Evolution 9: 347–348.

Bettin O., Cornejo C., Edwards P. J. & Holderegger R. (2007): Phylogeography of the high alpine *Senecio halleri* (*Asteraceae*) in the European Alps: in situ glacial survival with postglacial stepwise dispersal into peripheral areas. – Mol. Ecol. 16: 2517–2524.

Boža P. (1999): *Artemisia pancicii* (Janka) Ronniger subsp. *pancicii*. – In: Stevanović V. (ed.), Crvena knjiga flore Srbije 1. [Red Book of Flora of Serbia I], p. 141–142, 444, Ministarstvo za životnu sredinu R. Srbije, Biološki fakultet Univ. u Beogradu i Zavod za zaštitu prirode R. Srbije, Beograd.

Bremer K. (1994): *Asteraceae*. Cladistics and classification. – Timber Press, Portland.

Brewbaker J. L. (1957): Pollen cytology and self-incompatibility systems in plants. – J. Hered. 48: 271–277.

Brewbaker J. L. (1967): The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. – Am. J. Bot. 54: 1069–1083.

Bulová T. (2002): Biologie, ekologie a rozšíření druhů *Artemisia pancicii*, *Cardamine parviflora*, a *Gladiolus palustris* na Moravě [Biology, ecology and distribution of *Artemisia pancicii*, *Cardamine parviflora* and *Gladiolus palustris* in Moravia]. – Master thesis, Department of Botany and Zoology, Masaryk University, Brno.

Chiarugi A. (1926): Aposporia apogamia in *Artemisia nitida* Bertol. – Nuovo Giorn. Bot. Ital. 33: 501–626.

Clement M., Posada D. & Crandall K. A. (2000): TCS: a computer program to estimate gene genealogies. – Mol. Ecol. 9: 1657–1660.

Corander J., Marttinen P. & Mäntyniemi S. (2006): Bayesian identification of stock mixture from molecular marker data. – Fish Bull. 104: 550–558.

Charlesworth B. (1980): The cost of sex in relation to mating system. – J. Theor. Biol. 84: 655–671.

Chung M. G. & Kang S. S. (1996): Comparisons of genetic diversity in a narrow endemic, *Impatiens hypophylla*, and in its widespread congener, *I. textori* (*Balsaminaceae*). – Nord. J. Bot. 16: 359–365.

Crow J. F. & Aoki K. (1984): Group selection for a polygenic behavioral trait: estimating the degrees of population subdivision. – Proc. Natl. Acad. Sci. USA 81: 6073–6077.

Danihelka J. (1995): O druhu *Artemisia pancicii* v České republice a příbuzných taxonech [On the species *Artemisia pancicii* in the Czech Republic and related taxa]. – Zpr. Čes. Bot. Společ. 30: 81–90.

de Lange P. J. & Norton D. A. (2004): The ecology and conservation of *Kunzea sinclairii* (*Myrtaceae*), a naturally rare plant of rhyolitic rock outcrops. – Biol. Cons. 117: 49–59.

Dole J. (1992): Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (*Scrophularlaceae*). – Am. J. Bot. 79: 650–659.

Ehrendorfer F. (1964): Notizen zur Cytotaxonomie und Evolution der Gattung *Artemisia*. – Österr. Bot. Z. 111: 84–142.

Ehrich D. (2006): AFLPdat: a collection of R functions for convenient handling of AFLP data. – Mol. Ecol. Notes 6: 603–604.

Ehrich D., Gaudeul M., Assefa A., Koch M., Mummenhoff K., Nemomissa S., IntraBiodiv Consortium & Brochmann C. (2007): Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. – Mol. Ecol. 16: 2542–2559.

Eriksson O. (1996): Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. – Oikos 77: 248–258.

Ellmauer T. (ed.) (2005): Entwicklung von Kriterien, Indikatoren und Schwellenwerten zur Beurteilung des Erhaltungszustandes der Natura 2000-Schutzgüter. Band 4: Populäre Schutzobjekt-Steckbriefe. – Umweltbundesamt GmbH, Vienna.

Ellstrand N. C. & Elam D. R. (1993): Population genetic consequences of small population size: implications for plant conservation. – Annu. Rev. Ecol. Syst. 24: 217–242.

Evanno G., Regnaut S. & Goudet J. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Mol. Ecol. 14: 2611–2620.

Falush D., Stephens M. & Pritchard J. K. (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Mol. Ecol. Notes 7: 574–578.

Felsenstein J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. – Evolution 39: 783–791.

Fenster C. B. & Galloway L. F. (2000): Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (*Fabaceae*): consequences for conservation biology. – Cons. Biol. 14: 1406–1412.

Fischer M. & Matthies D. (1997): Mating structure, and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (*Gentianaceae*). – Am. J. Bot. 84: 1685–1692.

Friedman J. & Orshan G. (1975): The distribution, emergence and survival of seedlings of *Artemisia herba-alba* Asso, in the Negev desert of Israel, in relation to distance from adult plants. – J. Ecol. 63: 627–632.

Garcia A. A. F., Benchimol L. L., Barbosa A. M. M., Geraldi I. O., Souza Jr. C. L. & DeSouza A. P. (2004): Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. – Genet. Mol. Biol. 27: 1414–1427.

Gaston K. J. & Kunin W. E. (1997): Rare-common differences: an overview. – In: Kunin W. E. & Gaston K. J. (eds), The biology of rarity: causes and consequences of rare-common differences, p. 12–29, Chapman & Hall, London.

Gitzendanner M. A. & Soltis P. S. (2000): Patterns of genetic variation in rare and widespread plant congeners. – Am. J. Bot. 87: 783–792.

Gong X., Luan S. S., Hung K. H., Hwang C. C., Lin C. J., Chiang Y. C. & Chiang T. Y. (2011): Population structure of *Nouelia insignis* (*Asteraceae*), an endangered species in southwestern China, based on chloroplast DNA sequences: recent demographic shrinking. – J. Pl. Res. 124: 221–230.

Grulich V. (2004): *Artemisia pancicii* Danihelka et Marhold. – In: Slavík B. & Štěpánková J. (eds), Květena České republiky [Flora of the Czech Republic] 7: 172, Academia, Praha.

Hamrick J. L. & Godt M. J. W. (1989): Allozyme diversity in plant species. – In: Brown A. D. H., Clegg M. T., Kahler A. L. & Weir B. S. (eds), Plant population genetics, breeding, and genetic resources, p. 304–319, Sinauer, Sunderland.

Harrison S. & Hastings A. M. (1996): Genetic and evolutionary consequences of metapopulation structure. – Trends Ecol. Evol. 11: 180–183.

Harrison S., Viers J. H., Thorne J. H. & Grace J. B. (2008): Favorable environments and the persistence of naturally rare species. – Cons. Lett. 1: 65–74.

Hayat M. Q., Ashraf M., Khan M. A., Mahmood T., Ahmad M. & Jabeen S. (2009): Phylogeny of *Artemisia* L.: recent developments. – Afr. J. Biotechnol. 8: 2423–2428.

Hedrick P. W. (2001): Conservation genetics: where are we now? – Trends Ecol. Evol. 16: 629–636.

Holsinger K. E. (2000): Reproductive systems and evolution in vascular plants. – Proc. Natl. Acad. Sci. USA 97: 7037–7042.

Holub J. & Grulich V. (1999): *Artemisia pancicii* (Janka) Ronn. – In: Čeřovský J., Feráková V., Holub J., Maglocký S. & Procházka F. (eds), Červená kniha ohrožených a vzácných druhů rostlin a živočichů ČR a SR 5, Vyšší rostliny [Red data book of threatened plants and animals of the Czech Republic and Slovak Republic 5. Higher plants], p. 43, Príroda, Bratislava.

Honnay O., Coart E., Butaye J., Adriaens D., van Glabeke S. & Roldán-Ruiz I. (2006): Low impact of present and historical landscape configuration on the genetics of fragmented *Anthyllis vulneraria* populations. – Biol. Conserv. 127: 411–419.

Honnay O., Adriaens D., Coart E., Jacquemyn H. & Roldan-Ruiz I. (2007): Genetic diversity within and between remnant populations of the endangered calcareous grassland plant *Globularia bisnagarica* L. – Cons. Genet. 8: 293–303.

Huenneke L. F. (1991): Ecological implications of genetic variation in plant populations. – In: Falk D. A. & Holsinger K. E. (eds), Genetics and conservation of rare plants, p. 31–44, Oxford Univ. Press, Oxford.

Jäger E. J. (1987): Biologie, Chorologie und Ursachen des Reliktcharakters von *Artemisia laciniata* Willd. und *Artemisia rupestris* L. im herzynischen Gebiet. – Hercynia 24: 425–436.

Jakobsson M. & Rosenberg N. A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics 23: 1801–1806.

Jump A. S., Marchant R. & Peñuelas J. (2009): Environmental change and the option value of genetic diversity. – Trends Plant Sci. 14: 51–58.

Kitner M., Lebeda A., Doležalová I., Maras M., Křístková E., Beharav A., Nevo E., Pavlíček T. & Meglic V. (2008): AFLP analysis of *Lactuca saligna* germplasm collections from four European and three Middle East countries. – Israel J. Pl. Sci. 56: 185–193.

Korobkov A. A. (1992): Polyn' – *Artemisia* L. – In: Charkevič S. S. (ed.), Sosudistye rastenija sovetskogo Dal'nego Vostoka 6 [Vascular plants of the Soviet Far East 6], p. 120–161, Sankt-Petersburg.

Kučera J., Tremetsberger K., Vojta J. & Marhold K. (2008): Molecular study of the *Cardamine maritima* group (*Brassicaceae*) from the Balkan and Apennine Peninsulas based on amplified fragment length polymorphism. – Pl. Syst. Evol. 275: 193–207.

Kump B. & Javornik B. (1996): Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. – Pl. Sci. 114: 149–158.

Kunin W. E. & Gaston K. J. (1993): The biology of rarity: patterns, causes, and consequences. – Trends Ecol. Evol. 8: 298–301.

Lande R. (1993): Risks of population extinction from demographic and environmental stochasticity and random catastrophes. – Am. Nat. 142: 911–928.

Lienert J., Fischer M., Schneller J. & Diemer M. (2002): Isozyme variability of the wetland specialist *Swertia perennis* (*Gentianaceae*) in relation to habitat size, isolation, and plant fitness. – Am. J. Bot. 89: 801–811.

Luijten S. H., Kéry M., Oostermeijer J. G. B. & den Nijs H. C. M. (2002): Demographic consequences of inbreeding and out-breeding in *Arnica montana*: a field experiment. – J. Ecol. 90: 593–603.

Mantel N. (1967): The detection of disease clustering and a generalized regression approach. – Cancer Res. 27: 209–220.

McArthur E. D. & Plummer A. P. (1978): Biogeography and management of native western shrubs: a case study, section *Tridentatae* of *Artemisia*. – Great Basin Nat. Mem. 2: 229–243.

Meudt H. M. & Clarke A. C. (2007): Almost forgotten or latest practice? AFLP applications, analysis and advances. – Trends P. Sci. 12: 106–117.

Meusel H. & Jäger E. J. (1992): Vergleichende Chorologie der zentraleuropäischen Flora. Band III. – Gustav Fischer Verlag, Jena-Stuttgart, New York.

Mix C., Arens P. F, Rengelink R., Smulders M. J., van Groenendael J. M. & Ouborg N. J. (2006): Regional gene flow and population structure of the wind-dispersed plant species *Hypochaeris radicata* (*Asteraceae*) in an agricultural landscape. – Mol. Ecol. 15: 1749–1758.

Mráz P., Gaudeul M., Rioux D., Gielly L., Choler P. & Taberlet P. (2007): Genetic structure of *Hypochaeris uniflora* (*Asteraceae*) suggests vicariance in the Carpathians and rapid post-glacial colonization of the Alps from an Eastern Alpine refugium. – J. Biogeogr. 34: 2100–2114.

Nagylaki T. (1976): The evolution of one- and two-locus systems. – Genetics 83: 583–600.

Nagler M. (2010): Standorte und Vergesellschaftung von *Artemisia pancicii* in Österreich. – Diploma thesis, Faculty of Life Sciences, University of Vienna, Vienna.

Nei M. (1978): The theory of genetic distance and evolution of human races. – Japan. J. Human Genet. 23: 341–369.

Nordborg M., Hu T. T., Ishino Y., Jhaveri J., Toomajian C., Zheng H., Bakker E., Calabrese P., Gladstone J., Goyal R., Jakobsson M., Kim S., Morozov Y., Padhukasahasram B., Plagnol V., Rosenberg N. A., Shah C., Wall J. D., Wang J., Zhao K., Kalbfleisch T., Schulz V., Kreitman M. & Bergelson J. (2005): The pattern of polymorphism in *Arabidopsis thaliana*. – PLoS Biol. 3: e196.

Noyes R. D. (2007): Apomixis in the *Asteraceae*: diamonds in the rough. – Funct. Plant Sci. Biotechnol. 1: 207–222.

Oostermeijer J. G. B, Luijten S. H. & den Nijs J. C. M. (2003): Integrating demographic and genetic approaches in plant conservation. – Biol. Cons. 113: 389–398.

Page R. D. M. (1996): TREEVIEW: An application to display phylogenetic trees on personal computers. – Comp. Appl. Biosci. 12: 357–358.

Parker E. D. Jr. (1979): Ecological implications of clonal diversity in parthenogenetic morphospecies. – Am. Zool. 19: 753–762.

Paun O., Schönswetter P., Winkler M., IntraBioDiv Consortium & Tribsch A. (2008): Historical divergence versus contemporary gene flow: evolutionary history of the calcicole *Ranunculus alpestris* group (*Ranunculaceae*) in the European Alps and the Carpathians. – Mol. Ecol. 17: 4263–4275.

Pavlíček B. A., Hrdá S. & Flegr J. (1999): FreeTree: freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness: application in the RAPD analysis of the genus *Frenkelia*. – Folia Biol. 45: 97–99.

Peakall R. & Smouse P. E. (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – Mol. Ecol. Notes 6: 288–295.

Probatova N. S., Korobkov A. A., Gnutikov A. A., Rudyka E. G., Kotseruba V. V. & Seledets V. P. (2010): [Chromosome count for *Artemisia armeniaca*]. – In: Marhold K. & Breitwieser I. (eds), IAPT/IOPB chromosome data 10, Taxon 59: 1934–1938.

Rabinowitz D. (1981): Seven forms of rarity. – In: Synge H. (ed.), The biological aspects of rare plant conservation, p. 205–217, John Wiley & Sons, New York.

Rahimmalek M., Tabatabaei B. E. S., Arzani A. & Etemadi N. (2009): Assessment of genetic diversity among and within *Achillea* species using amplified fragment length polymorphism (AFLP). – Biochem. Syst. Ecol. 37: 354–361.

Richards A. J. (1986): Plant breeding systems. – George Allen & Unwin, London.

Rohlf F. J. (1998): NTSYS-pc. Numerical taxonomy and multivariate analysis system. – Applied Biostatistics Inc., New York.

Rosenberg N. A. (2004): Distruct: a program for the graphical display of population structure. – Mol. Ecol. Notes 4: 137–138.

Rotreklová O., Bureš P. & Grulich V. (2004): Chromosome numbers for some species of vascular plants from Europe. – Biologia 59: 425–433.

Russell R. J., Fuller J. D., Macaulay M., Hats B. G., Jahoor A., Powell W. & Waugh R. (1997): Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. – Theor. Appl. Genet. 95: 714–722.

Schaal B. A. & Leverich W. J. (1996): Molecular variation in isolated plant populations. – Pl. Spec. Biol. 11: 33–40.

Schaffer M. L. (1987): Minimum viable populations: coping with uncertainty. – In: Soulé M. E. (ed.), Viable populations for conservation, p. 69–86, Cambridge Univ. Press, Cambridge.

Schemske D. S., Husband B. C., Ruckelshaus M. H., Goodvillie C., Parker I. M. & Bishop J. C. (1994): Evaluating approaches to the conservation of rare and endangered plants. – Ecology 75: 584–606.

Schmidt K. & Jensen K. (2000): Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (*Scrophulariaceae*) and its relation to population size and reproductive components. – Am. J. Bot. 87: 678–689.

Slatkin M. (1987): Gene flow and the geographic structure of natural populations. – Science 236: 787–792.

Schönswetter P. & Tribsch A. (2005): Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (*Apiaceae*). – Taxon 54: 725–732.

Taberlet P., Gielly L., Pautou G. & Bouvet J. (1991): Universal primers for amplification of three non-coding regions of chloroplast DNA. – Pl. Mol. Biol. 17: 1105–1109.

Tamura K., Dudley J., Nei M. & Kumar S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. – Mol. Biol. Evol. 24: 1596–1599.

Thines M., Runge F., Telle S. & Voglmayr H. (2010): Phylogenetic investigations in the downy mildew genus *Bremia* reveal several distinct lineages and a species with a presumably exceptional wide host range. – Eur. J. Pl. Pathol. 128: 81–89.

Thompson J. D. (1999): Population differentiation in Mediterranean plants: insights into colonization history and the evolution and conservation of endemic species. – Heredity 82: 229–236.

Torre A., Lopez S., Yglesias E. & Cornelius J. P. (2008): Genetic (AFLP) diversity of nine *Cedrela odorata* populations in Madre de Dios, southern Peruvian Amazon. – For. Ecol. Manag. 255: 334–339.

Torrell M., Bosch M., Martín J. & Vallès J. (1999): Cytogenetic and isozymic characterization of the narrow endemic species *Artemisisa molinieri* (*Asteraceae*, Anthemideae): implications for its systematics and conservation. – Can. J. Bot. 77: 51–60.

Torrell M., Vallès J., Garcia-Jacas N., Mozaffarian V. & Gabrielian E. (2001): New or rare chromosome counts in the genus *Artemisia* L. (*Asteraceae*, Anthemideae) from Armenia and Iran. – Bot. J. Linn. Soc. 135: 51–60.

Tutin T. G., Heywood V. H., Burges N. A., Moore D. M.,Valentine D. H., Walters S. M. & Webb D. A. (eds) (1976): Flora Europaea, Volume 4: *Plantaginaceae* to *Compositae* (and *Rubiaceae*). – Cambridge Univ. Press, Cambridge.

Vallès J. & McArthur E. D. (2001): *Artemisia* systematics and phylogeny: cytogenetic and molecular insights. – In: McArthur E. D. & Fairbanks D. J. (eds), Proceedings of Shrubland ecosystem genetics and biodiversity, 13–15 June 2000, Provo, Utah, p. 67–74, US Department of Agriculture Forest Service, Rocky Mountain Research Station, Ogden.

Vallejo-Marín M. & O'Brien H. E. (2007): Correlated evolution of self-incompatibility and clonal reproduction in *Solanum* (*Solanaceae*). – New Phytol. 173: 415–421.

van Dijk P. J. (2003): Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. – Phil. Trans. R. Soc. London B358: 1113–1120.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucl. Acids Res. 23: 4407–4414.

Wang W., Chen L., Yang P., Hou L., He C., Gu Z. & Liu Z. (2007): Assessing genetic diversity of populations of topmouth culter (*Culter alburnus*) in China using AFLP markers. – Biochem. Syst. Ecol. 35: 662–669.

Waser N. M. & Price M. V. (1994): Crossing-distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness. – Evolution 48: 842–852.

Wendelberger G. (1959): Die mitteleuropäischen Reliktvorkommen der *Artemisia*-Arten aus der Sektion Heterophyllae. – Verh. Zool.-Bot. Ges. Wien 98/99: 57–95.

Wendelberger G. (1960): Die Sektion *Heterophyllae* der Gattung *Artemisia*. – Bibliotheca Botanica 125, Stuttgart.

Weising K., Nybom H., Wolff K. & Günter K. (2005): DNA Fingerprinting in plants principles, methods, and applications, Ed. 2. – CRC Press, Boca Raton.

Whitacre M. K. & Call C. A. (2006): Recovery and germinability of native seed fed to cattle. – West. N. Am. Nat. 66: 121–128.

Wright S. (1951): The genetic structure of populations. – Ann. Eugen. 15: 323–354.

Wróblewska A., Brzosko E., Chudzińska E., Bordács S. & Prokopiv A. I. (2010): Cytotype distribution and colonization history of the steppe plant *Iris aphylla*. – Ann. Bot. Fennici 47: 23–33.

Young J., Longland W. & Blank R. (1996): Management of woody species of *Artemisia* in western North America. – In: Caligari P. & Hind D. (eds), Compositae: biology and utilization, p. 271–290, Royal Botanic Gardens, Kew.

Received 2 July 2010
Revision received 19 May 2011
Accepted 23 May 2011