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Genetic variation within the endangered species *Aldrovanda vesiculosa* (Droseraceae) as revealed by RAPD analysis

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Abstract

Aldrovanda vesiculosa L. (Droseraceae) is an endangered aquatic carnivorous plant species inhabiting standing dystrophic waters across Europe, Asia, Australia, and Africa. Despite its widespread occurrence, its population is patchily distributed. Proliferation in these mostly isolated habitats is largely clonal by shoot branching. We assessed the level of variation of random amplified polymorphic DNAs (RAPDs) in a collection of plants from Europe, Asia, and Australia to study the distribution of genetic variation. The low level of genetic variation found in a previous allozyme study was confirmed in that only 14% of 151 RAPD primers gave polymorphic banding patterns. The proportion of polymorphic bands over all primers was 37% with a mean Jaccard distance of 0.62. Cluster analysis and ordination analysis identified three clusters of closely related plants: an Australian and Japanese accession, a Ukrainian, Russian, and Rumanian accession, and a third accession from Poland and Germany. Although the plants from Germany and NW Australia did not differ from other members of the same RAPD cluster on the basis of morphological or physiological characteristics, these plants were clearly of recombinant origin based on the results of compatibility tests. Remaining accessions possessed RAPD patterns consistent with predominantly asexual mode of reproduction. By focussing on the modes of reproduction in combination with physiological and

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morphological features, the RAPD analyses can be used for restoration strategies of endangered plant species.

Keywords: *Aldrovanda vesiculosa*; Compatibility test; Genetic variation; RAPD

1. Introduction

Aldrovanda vesiculosa L. (Droseraceae) is a critically endangered aquatic carnivorous plant. Although its range extends across a vast territory on all continents of the Old World, from north Russia to SW Australia, its recent occurrence is patchy in distribution. In most European countries, it is undergoing local extinction (Adamec, 1995). Its rootless, free-floating shoots grow just below the surface in shallow standing, dystrophic waters (Huber, 1961; Adamec, 1995). Temperate Euro-Asian populations differ from (sub)tropical Australian ones in that the former produce morphologically distinct and highly dormant winter buds (turions) and do not contain the pigment anthocyanin, while the latter produce only non-dormant winter shoot apices and contain anthocyanin (Adamec, 1999a).

The origin of the recent populations of *A. vesiculosa* in Europe is unclear (Berta, 1961; Huber, 1961; Adamec and Tichý, 1997). According to Yakubovskaya (1991), they represent a tertiary relict, while other investigators suggest post-glacial migration of African plants to the European continent (Berta, 1961; Huber, 1961). The post-glacial spread of *A. vesiculosa* is hypothesized to have been highly unequal in time and space and probably reflects dispersal along the migration routes of water birds (Berta, 1961; Huber, 1961). The European plants rarely flower and the production of viable seeds is exceptional (Adamec and Tichý, 1997; Adamec and Lev, 1999). Instead, these plants predominantly propagate by means of vegetative reproduction via apical branching of the shoots (Adamec, 1995, 1999b).

A unique world-wide collection of *A. vesiculosa* is kept at the Institute of Botany, in Třeboň, Czech Republic (Adamec, 1997a,b). It comprises plants originating from Europe, Japan, and Australia. The availability of cultivated plants from the three continents allowed us to estimate the genetic variation within this endangered species, using molecular tools.

Preliminary results obtained from a study of allozymes suggest very low genetic diversity among European populations (Adamec and Tichý, 1997). No variation was found among plants from NE Poland, east Poland, and south Germany at 15 loci of seven enzymatic systems that were tested. Only one locus, NADH dehydrogenase, was missing in all plants from NE Poland. Interpretation of allozymes as markers of silent divergence is constrained by a limited number of marker loci and their possible non-neutral character, because they code for functional enzymes. Consequently, the low level of polymorphism at the allozyme loci could be explained by natural selection preferring a narrow range of genotypes of this highly specialized, stenotopic (with narrow ecological requirements) plant species. Alternatively, a recent origin of the *Aldrovanda* accessions, perhaps in combination with bottleneck(s) during the recent evolutionary history of this taxon, are plausible explanations of the low level of genetic variation.

In this study, we use random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990; Welsh and McClelland, 1990) to study polymorphism in *A. vesiculosa*. Despite

some limitations—a lower reproducibility relative to allozymes, dominant nature of the markers, and the susceptibility to contamination (Lynch and Milligan, 1994)—RAPD analyses often reveal large amount of variation at both the population and species levels. Decanucleotid primers used in RAPD-PCR can find targets in any DNA (of fungal, algal, or even insect origin). As submersed aquatic plants frequently contain epiphytic or endophytic algae, which cannot be removed easily, special attention should be paid to this source of error. In spite of this potential complication, several aquatic plant species have been successfully investigated with RAPD markers (Madeira et al., 1997; Mader et al., 1998). The principal goal of this study is to examine genetic variation among accessions of *A. vesiculosa* from Europe, Asia, and Australia using RAPD method and to provide a comparison to the extremely low level of genetic diversity based on the allozyme loci. Results from this investigation will be useful for the selection of the most suitable genotypes for (re)introduction projects and for determination of the origin of particular populations of this and other aquatic species that show fragmented distributions.

2. Material and methods

2.1. Plant material

A. vesiculosa plants from eight European, one Japanese, and three Australian populations were used for analyses (Table 1). Individual plants from east Poland were grown outdoors in a 1 m^2 plastic container at the Institute of Botany at Třeboň (Adamec, 1997a,b). Plants from all other populations were grown outdoors in 3–20 l aquaria, which stood in a 2.5 m^2 plastic container filled with water for cooling (Adamec and Tichý, 1997; Adamec, 1999a). In addition, plants from all the three Australian populations were also grown in small aquaria indoors (Adamec, 1999a). As plants from each population were grown separately in covered aquaria, any incidental mixing of the individuals was excluded. Plants in all cultures propagated strictly vegetatively.

In vitro cultures of the plants from east Poland and Japan were prepared from seeds in 1995 and grown in a Gamborg B5 liquid medium (Kondo et al., 1997). Since 2000, they have been cultivated in a modified Gamborg medium at 25–28 °C under fluorescent illumination (Adamec and Pásek, 2000). In 1999, in vitro cultures of the plants from SE and north Australia were prepared separately for each accession. After surface sterilization by 0.5% NaClO (30–60 s), tissue was collected from shoot apices. The cultivation medium and the growth conditions were the same as in the previous case.

2.2. DNA extraction

Plants from both indoor and outdoor cultures were thoroughly washed with water to remove sessile organisms and plant traps with prey were excised. Several shoots from an accession were pooled together. Only very clean apical shoots 3–6 cm long were used for DNA extraction as described by Štorchová et al. (2000) with little modifications. Briefly, about 200 mg of fresh shoot tissue was ground in liquid nitrogen, transferred to the extraction buffer (0.34 M sorbitol, 0.1 M Tris–HCl pH 7.6, 5 mM EDTA, 0.2% (v/v) 2-mercaptoethanol) and

Table 1
Aldrovanda vesiculosa accessions from Europe, Asia and Australia

Abbreviation	Country of origin	Site of collection	Latitude	Longitude (°E)	Year of collection	No. of collected plants	Start of cultivation at Třeboň	No. of stocked plants	Reference
EP	East Poland	Lake Długie, Łeczna-Włodawa Lake District	51°26′N	23°06′	1993	31	1993	31	Kamiński (1987)
NEP	NE Poland	Lake Kruglak, Augustów-Suwalki Lake District	53°55′N	23°00′	1998	15	1998	15	Kamiński (1987)
WP	West Poland	Lake Ostrowo, Miedzychodzko-Sierakowskie Lake District	52°33′N	16°11′	1994	5	1994	5	Gramsz and Jasińska (1986)
NR	North Russia	Shallow lake near the estuary of the Sviri river on the southeastern bank of Lake Ladoga	60°29′N	32°57′	1997	8	1997	8	Afanas'ev (1953)
UK	North Ukraine	Western bank of the Kiev reservoir (on the Dn'epr river) near Stracholes' village, at the T'et'erev river estuary	51°03′N	30°25′	1997	6	1997	6	Adamec (1995)
RO	Rumania	Shallow lake on Obretin island near the Sulina branch in the Danube delta	45°11′N	29°19′	1998	20	1998	20	–
GR	Germany	Artificial site, Lake Mettmenhaslisee near Kloten in the Zurich Canton; from native site, pool Bűhlweiher at Lake Constance near Lindau, SW Bavaria, Germany	47°34′N	9°41′	1994	2	1994	2	Koch (1950); Adamec (1995)
IT	Italy	A swamp close to Lake Sihlsee near Einsiedeln, Switzerland. Plant origin from north Italy?	46°30′N	11°20′	1980s	?	1993	3	–
JP	Japan	Hozoji pond near Hanyu city in Saitama Prefecture, Honshu island	36°12′N	139°42′	1980s	?	1998	12	Komiya (1966)
SEA	SE Australia	Longvale Swamp near Broulee at Batemans Bay at the East Coast, NSW	35°35′S	150°09′	1997	12	1997	12	Adamec (1999a)
NA	North Australia	Girraween Lagoon, ca. 30 km SE of Darwin, NT	12°31′S	131°05′	1994	2	1998	3	Wilson (1995); Adamec (1999a)
NWA	NW Australia	Billabong near Mertens Creek, Big Mertens Falls, Kimberley, WA	14°50′S	125°41′	1990s	?	1999	2	Lowrie (1998)

centrifuged at 13 000 rpm for 10 min. The pellet was suspended in the extraction buffer, the same volume of the lysis buffer (0.2 M Tris–HCl pH 7.6, 2 M NaCl, 0.05 M EDTA, 2% CTAB) was added, then chloroform extraction, isopropanol precipitation and washing with 80% ethanol were performed. If necessary, shoots were frozen in liquid nitrogen and stored in deep-freeze (−70 °C) for later DNA extraction. Stems preserved in a saturated NaCl solution with 2.6% of CTAB (Rogstad, 1992) were stored in freeze (−20 °C) for several months, then DNA was extracted exactly as described in Štorchová et al. (2000) without liquid nitrogen.

2.3. RAPD

The PCR reaction was performed using a T-Gradient cycler (Biometra). For each sample, at least two PCR reactions per primer were done. Three or six nanograms of purified genomic DNA per reaction were used in a final volume of 25 μl under the following conditions: 200 μM of each dNTP (MBI Fermentas), 3.0 mM $MgCl_2$, 0.48 μM primer, magnesium-free reaction buffer and 1 U *Taq* DNA polymerase (Promega). After initial heating for 5 min at 94 °C, samples were PCR amplified using 40 cycles (94 °C, 20 s; 42 °C, 20 s; 72 °C, 1 min) followed by a final extension of the PCR products for 4 min at 72 °C. The products of amplification were analyzed by electrophoresis in 1.6–2.0% agarose gels with 1× TAE running buffer (0.04 M Tris–acetate, 0.001 M EDTA, pH 8.0), visualized by ethidium bromide staining, and photographed under UV light with a digital Kodak camera.

Ten oligonucleotides from the kit A (Advanced Biotechnologies) and 141 primers from the University of British Columbia, Vancouver (UBC 1–5, 21–25, 31–40, 80–100, and 301–400), 151 primers in total, were tested for amplification and polymorphism using DNA from two individuals (east Poland, north Australia). Forty-three primers yielded reproducible bands; 21 of them gave polymorphic markers (Table 2).

2.4. Data analysis

Only the RAPD patterns resulting from the same experiment (PCR reaction mixture and electrophoresis gel) were compared. Polymorphic, reproducible amplification products were scored as present (1) or absent (0); ambiguous and monomorphic bands were excluded. RAPD fragments present in outdoor cultures that were absent from in vitro lineages were excluded from the analyses. Genetic diversity was estimated by the Shannon index (Lewontin, 1972):

$$H = -\sum_{i=1}^{k} p_i \ln p_i$$

where k is the number of RAPD bands produced with the respective primer, and p_i is the frequency of the ith fragment.

To further investigate phenetic relationships among accessions, the binary RAPD matrix was used to compute Nei–Li (Nei and Li, 1979) and Jaccard (1908) similarity coefficients. A neighbor-joining (NJ) tree was built from the matrix of Jaccard coefficients of dissimilarity using the FreeTree software (Pavlíček et al., 1999). The original dataset was resampled

Table 2
Sequences and codes of random decamers and the number of polymorphic fragments amplified

Primer	Sequence	Total no. of bands	Polymorphic bands	Polymorphic bands (%)
ABA 04	AAT CGG GCT G	9	4	44.4
ABA 20	GTT GCT ATC C	6	2	33.3
UBC 01	CCT GGG CTT C	4	2	50.0
UBC 25	ACA GGG CTC A	7	6	85.7
UBC 33	CCG GCT GGA A	3	1	33.3
UBC 83	GGG CTC GTG G	8	2	25.0
UBC 84	GGG CGC GAG T	4	1	25.0
UBC 90	GGG GGT TAG G	5	1	20.0
UBC 322	GCC GCT ACT A	8	5	62.5
UBC 337	TCC CGA ACC G	6	4	66.6
UBC 341	CTG GGG CCG T	15	9	60.0
UBC 347	TTG CTT GGC G	9	7	77.7
UBC 353	TGG GCT CGC T	7	5	71.4
UBC 354	CTA GAG GCC G	5	3	60.0
UBC 365	TAG ACA GAG G	4	2	50.0
UBC 368	ACT TGT GCG G	4	2	50.0
UBC 375	CCG GAC ACG A	7	2	28.7
UBC 382	ATA CAC CAG C	6	4	66.7
UBC 383	GAG GCG CTG C	10	10	100.0
UBC 388	CGG TCG CGT C	8	5	62.5
UBC 392	CCT GGT GGT T	4	2	50.0
Total		213	79	53.0

(1000 replicates) and bootstrap support values (%) for the nodes were calculated. Principal coordinate analysis (PCoA) was computed by SYN-TAX 2000 (Podani, 2001) using the Jaccard dissimilarity coefficients. Object coordinates were stored and three-dimensional graph was visualized using SAS 8.1 (SAS Institute).

Recombinants can be identified using character incompatibility (Wilkinson, 1995; Mes, 1998; Van der Hulst et al., 2000; Ceplitis, 2001; Mes et al., 2002). These approaches are suited to detect recombinants when using anonymous markers that are distributed over the entire genome. Binary markers cannot represent a tree-like relationship if many markers display all the four possible combinations when two markers are present (i.e. 0–0, 0–1, 1–0, and 1–1). The level of disagreement contributed by individual genotypes can be determined by calculating the contribution of an individual RAPD pattern to the number of incompatible marker combinations in a dataset, and by contrasting this number to a dataset that lacks the genotype of interest (Wilkinson, 1995). The probability of the number of conflicts contributed by a genotype can be assessed by permutation of the markers of a genotype using the empirical marker frequencies, followed by recalculation of the number of conflicts. By repeating this procedure a large number of times, the resulting distribution of conflicts can be used to determine the probability of the conflict of genotypes by permutation-tail probabilities (Wilkinson, 1995). We used this technique to assess the likelihood of recombinant origin among our accessions tested against the random expectation generated by performing 10 000 permutations of the dataset.

3. Results

3.1. Analysis of genetic diversity

Forty-three of 151 primers generated a total of 213 reproducible bands. The primers with higher G + C content resulted in better amplification—the average G + C content of 43 useful primers was 69.8%, in contrast to 65.9% of the whole set of 151 primers. Twenty-one primers (14% of all tested primers) produced 79 polymorphic markers (37% of all markers) (Table 2). The average proportion of polymorphic markers across primers was 53%, ranging between 20% (UBC 90) and 100% (UBC 383). Of the 79 informative fragments, 39 (49%) were exclusive to only one accession. The highest number of exclusive fragments (14) was present in NW Australia, followed by Rumania (9) and Germany (7). The remaining nine exclusive fragments were distributed among SE Australia (2), east Poland (2), Russia (2), west Poland (1), Japan (1) and NE Poland (1). The large number of exclusive markers account for a substantial portion of the genetic diversity as illustrated by the mean Shannon index (per primer 0.920), whereas the mean Shannon index calculated only from frequencies of non-exclusive markers was 0.516 (Table 3).

DNAs from four in vitro lineages (east Poland, Japan, SE Australia, north Australia) were included in the same RAPD experiment together with DNAs from the four corresponding outdoor cultures of *A. vesiculosa*. Banding patterns of outdoor and in vitro plants from Japan were completely identical, while outdoor cultures from SE and north Australia had one additional band relative to their in vitro counterparts. RAPDs of in vitro and outdoor cultures from east Poland differed in two bands. The differences between outdoor and in vitro RAPD patterns ranged from 0 to 2.2%, with outdoor samples always having extra bands when compared to the corresponding in vitro samples. These RAPD fragments may have originated from contaminating DNA, e.g. from endophytic algae, which cannot be removed by washing. Alternatively, they may reflect very limited intra-population variation, as the outdoor cultures in Třeboň's collection were established from various numbers of stocked plants (Table 1). East Poland in vitro lineage was derived from seed, and thus, has undergone recombination, which could explain the two bands difference compared to its outdoor counterpart. The minute differences between RAPD patterns, obtained from plants grown under sterile and field conditions, nearly exclude the possibility of artefactual origin of exclusive markers.

3.2. Phenetic relationships and compatibility tests

To further investigate relationships among accessions, a cluster analysis was performed on the matrix of Nei–Li (1979) similarity coefficients. An unrooted neighbor-joining tree

Table 3
Comparison of genetic diversity and dissimilarity coefficients among 12 accessions of *Aldrovanda vesiculosa* calculated from the dataset with or without exclusive markers

	No. of markers	Shannon index (per primer)	Mean Nei–Li (S.D.)	Mean Jaccard (S.D.)
All polymorphic markers	79	0.920	0.534 (0.179)	0.615 (0.177)
Non-exclusive polymorphic markers	40	0.516	0.394 (0.155)	0.548 (0.164)

S.D.: standard deviation.

(Fig. 1) clearly discriminates Australian accessions from the others. The accession from NW Australia is genetically most differentiated from all other accessions. The topology of the European accessions is not strongly supported, as the majority of nodes have low bootstrap support. Only the group comprising Russia, Ukraine, and Rumania is somewhat supported (bootstrap support 58%).

The general pattern of relatedness marginally supported in the NJ tree is reaffirmed by the PCoA results (Fig. 2). The three principal axes (explaining 22, 16, and 13% of the total variance, respectively), revealed two distinct groups—Australian and Euro-Asian. The Japanese accession was closest to the Australian accessions.

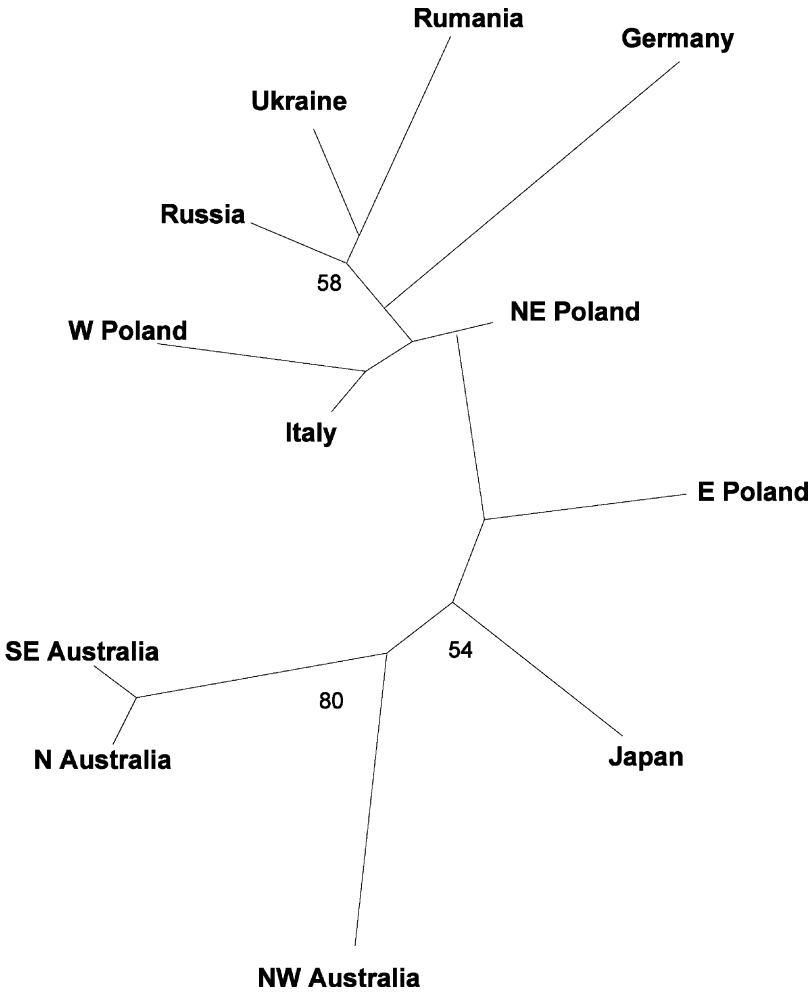

Fig. 1. An unrooted neighbor-joining tree, showing relationships between accessions of *Aldrovanda vesiculosa*, based on the matrix of Jaccard coefficients of dissimilarity calculated from RAPD dataset. Bootstrap support values for each branch are indicated as percentages.

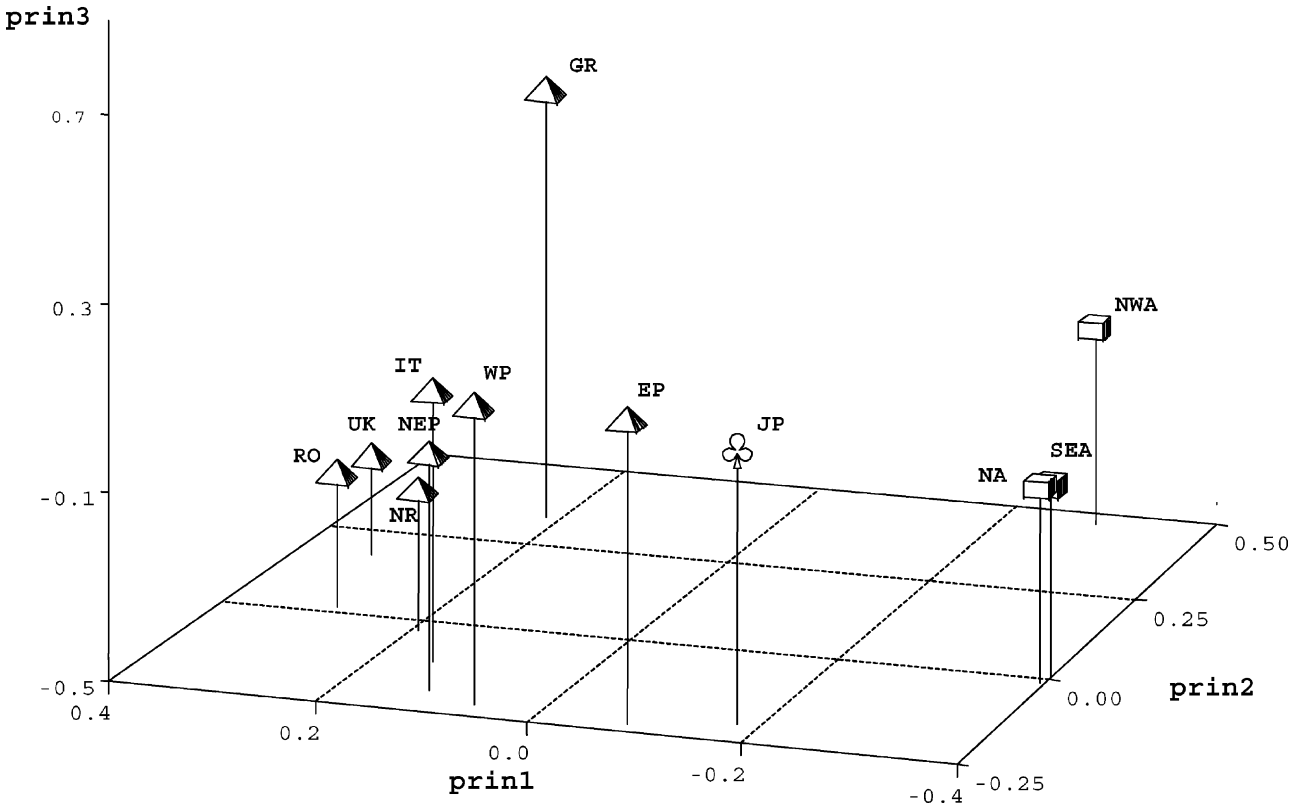

Fig. 2. Three-dimensional representation of principal coordinate analysis of phenetic relationships between accessions of *Aldrovanda vesiculosa*. Percentage of total variance for RAPD dataset, explained by the corresponding axis, was: prin1 22%, prin2 16%, prin3 13%.

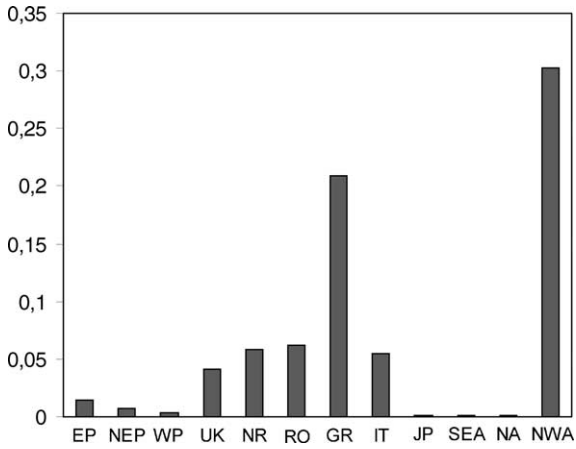

Fig. 3. Probability of incompatibility of the RAPD patterns of accessions of *Aldrovanda vesiculosa* based on 10 000 permutations.

One explanation for a lack of strong support among groups is that some of the accessions are of hybrid origin. Unlike other phylogeny building techniques (e.g. neighbor-joining trees), PCoA does not assume strict branching patterns, which is appropriate when recombinants are involved. Although hybrid accessions are, in principal, detectable using PCoA, an intermediate position of hybrid plants can only confidently be assessed when putative parental plants are known. This is not the case for the *Aldrovanda* accessions. Consequently, the RAPD dataset was analyzed for signatures of past recombination events using compatibility tests that do not depend on this assumption. There are only two accessions that were not significantly different from random genotypes, i.e. south Germany and NW Australia (Fig. 3). Because they differ considerably in the distribution of the informative RAPD markers following randomization tests, we conclude that they result from a recombinant event. If these accessions are deleted from the dataset, some of the branches clearly show increased bootstrap support using neighbor-joining distance analysis (68% when compared to 54% for the Japanese and Australian plants in the entire dataset). In addition, the grouping of the Ukrainian, Russian, and Rumanian accessions gained support of 77%. This contrasts with the 58% support for this internode when using the entire dataset. Thus, the identification of the two recombinant accessions results in increased support for the groupings of our *Aldrovanda* collection.

4. Discussion

4.1. Low genetic variation in A. vesiculosa

This study provides the first characterization of molecular genetic DNA diversity among populations of *A. vesiculosa*, an endangered aquatic plant with a patchy distribution. It also highlights the utility of using RAPD markers as an efficient and inexpensive tool

for conservation geneticists when reproducibility and outside contamination are taken into account.

The low genetic diversity among the accessions of *A. vesiculosa* from Europe, Japan and Australia is evident from the fact that 151 primers were required to obtain 21 primers with 79 polymorphic markers. The proportion of polymorphisms was only 37%, with a mean genetic distance based on Nei–Li and Jaccard coefficients of dissimilarity of 0.534 and 0.615, respectively (Table 3). This corresponds to the finding that genetic variation in aquatic angiosperms is generally low (Laushman, 1993), which might be due to a variety of causes, such as the uniform nature of the habitat, high frequency of asexual reproduction, and long-distance dispersal of seeds or shoots.

However, two RAPD studies, that sampled aquatic plants across large geographic areas, found much higher levels of genetic diversity. Mader et al. (1998) tested 20 primers, which provided 99 (60.4%) polymorphic markers in *Potamogeton pectinatus* L. from Europe, America, Siberia and northern Africa. Madeira et al. (1997) used five primers (selected from 60), which produced 85 polymorphic bands in *Hydrilla verticillata* (L.f.) Royle, an aquatic weed from Asia, Australia and America.

A. vesiculosa differs from these two species in being a very rare, stenotopic carnivorous species. It persisted through the last glaciation in one or several refuges, from which it has migrated to the recent localities (Adamec, 1995). As suitable habitats, shallow dystrophic waters, were gradually disappearing, populations experienced repeated bottleneck events. This history contrasts with the fast, recent spread resulting in large, more continuous population sizes in *P. pectinatus* or *H. verticillata*. Populations of *A. vesiculosa* are small, seed set is low, and germination infrequent in temperate populations. If sexual reproduction is rare, mutations represent the only force that can cause genetic differentiation between parents and offspring. The high proportion of exclusive markers—49% of all informative markers—can be explained by the accumulation of mutations in distant, isolated populations with low or absent gene flow among them. Supposing that *A. vesiculosa* has gone through recent bottlenecks, there has been no time for mutations to create considerable genetic variation.

This conclusion is in agreement with the drift causing a decrease in genetic variation described in other rare plant species. No polymorphic marker was found among 131 RAPD bands produced by 11 different decamers in *Limonium cavanillesii* Herben, a plant species endemic to the east Mediterranean region of Spain (Palacios and Gonzales-Candelas, 1997). Thirteen of 20 random primers (65%) generated 60 (20.8%) polymorphic bands in *Amenthotaxus formosana* Li, a tree species endemic to Taiwan (Wang et al., 1996). However, the distribution area of rare species is often small, restricted to one island, a mountain ridge, or a part of the coast. To our knowledge, our study is the first one that describes a similar low level of genetic variation among individuals sampled from such a large geographic area.

4.2. Phenetic relationships and mode of reproduction

The results of multivariate analyses that clearly separated Australian and European accessions with the Japanese accession as an intermediate, correlate well with the morphological and physiological differences observed during cultivation. All three Australian accessions display high anthocyanin expression, while all Euro-Asian plants lack anthocyanin (Adamec, 1999a). Moreover, there are conspicuous differences between these two

groups of *Aldrovanda* accessions in winter bud (turion) size and dormancy, in the characteristics of branching and formation of axillary buds, and in the boron requirement for growth (Adamec, 1997b, 1999a). Within the three Australian accessions, only minor differences are found.

The European accessions form a group in NJ tree, which has an unclear structure, with only one cluster significantly supported (Rumania, north Russia and Ukraine). This grouping corresponds to morphological features. Within the group of eight Euro-Asian populations, only the plants from north Russia and Rumania differ phenotypically from the others. Outdoor-grown plants from north Russia form turions 2–4 weeks sooner than the other populations (Adamec, 1999c). Under comparable cultivation conditions, the Rumanian plants are more robust, flower more frequently, and have differently shaped ripe capsules (Adamec, 1999d).

The identification of potential recombinants by means of compatibility tests represents a promising approach to aid conservation strategies of endangered species. The NW Australian and German accessions did not differ from the other accessions in their respective clusters based on RAPDs, but contrary to the other plants, there was strong support for a recombinant origin of these plants. This interpretation receives strong support from the increased levels of bootstrap support when the two recombinant accessions were removed from the dataset.

Accessions of *Aldrovanda* from NW Australia and Germany are clearly indicated to be recombinants in the collection of *Aldrovanda* accessions that were investigated in this study. Although they did not differ in morphological or physiological characters from the other accessions of the same cluster (Adamec, 1999a,c), they have signatures of sexual reproduction in their recent evolutionary history. Given the low level of genetic differentiation among accessions of *Aldrovanda*, these signatures are readily detectable. The identification of recombinants can be used via artificial breeding schemes as an important means to maintain or restore the genetic variability of endangered plant species. In contrast, RAPD markers of the remaining accessions are in agreement with the prevailing asexual mode of reproduction.

Definite conclusions regarding the recent evolution of this carnivorous species must await inclusion of African accessions, which constitutes the probable cradle of *Aldrovanda*. The analysis of African populations by RAPD or other suitable method (AFLP, microsatellites) would answer the questions about the origin of European populations and hypotheses regarding post-glacial migration routes of this very rare plant species.

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